Journal of Agricultural Science
http://www.ccsenet.org/jas
Vol. 2, No. 1
March 2010

Seasonal Abundance of Onion thrips, *Thrips Tabaci* Lindeman. in Sokoto, Nigeria

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Abstract

Onion seedlings were transplanted from November to March to study the population dynamics of onion thrips, *Thrips tabaci.* There are four transplants in 2001/2002 and five in 2002/2003 seasons. In 2000/2001 season onion plants were grown in exploratory trials and in 2003/2004, water traps were used to confirm results of the previous experiments. Results indicate that November transplant had a peak population of onion thrips in late February (176 thrips/plant); December (416 thrips/plant) and January (608 thrips/plant) transplants peaked in March, and February (148 thrips/plant) and March (86) transplants had peaks in April. Water traps indicate that the peak population of adult thrips was at the time of harvest in April, similar to November transplant. The early transplant (November) had peak thrips population at maturity and middle transplant recorded the peak population middle of the season and late transplant had their peaks early part of the growing season. Therefore, the findings of this work revealed that onion thrips in Sokoto, Nigeria, breed from January to May with peak in March.

Keywords: Breed, Exploratory, Peak, Season, Seedlings, *Thrips tabaci,* Transplant

1. Introduction

1.1 Importance of Onion

Edible alliums are important vegetables worldwide. In terms of global weight of vegetables produced, at nearly 28 million tonnes per annum, only tomatoes and cabbage exceed bulb onions in importance (FAO, 1991). The distinctive flavour of onions is appreciated by people. One of the advantages of onion is that the bulbs can be harvested and sold "green" for salads (Lannoy, 2001), while the mature bulbs are cooked or eaten raw as vegetable (Straub and Emmett, 1992).

1.2 Onion Thrips

Onions and related Allium crops are subject to a variety of diseases and attack by arthropod pests that can reduce crop yield and quality (Lorbeer *et al.*, 2002). Probably the most damaging pests worldwide are the insignificant looking thrips or thunderflies. These are slender insects only about 2 mm long as adults. They are found wherever alliums are grown, but are most severe in the warmer production regions (Brewster, 1994). Soni and Ellis (1990) listed seven species of Thrips as allium pests, the best known of which is *Thrips tabaci*, the onion thrips, which attacks all edible allium. Onion thrips have a wide range and populations move from one crop to another when conditions change, such as when neighbouring crops are harvested (Shelton and North, 1986). Thus, the temporal and spatial arrival of onion thrips population into onion fields is variable and relatively unpredictable (Gangaloff, 1999).

According to Kranz *et al*. (1977), the number of thrips on a crop can increase rapidly in dry weather and decrease rapidly after rain. They found that large number of thrips attacking a crop at the seedling stage could cause severe or even total losses with onion, cabbage or cotton. However, once established and growing vigorously, most plants could tolerate feeding damage. Adults and nymphs were present from February to harvest (April or May) with peak abundance in early April (Edelson *et al*., 1986). Kranz *et al*. (1977) reported that control of alternative host plants is unlikely to be a useful method except under exceptional circumstances, because of wide host range. The crop may be protected by bringing forward planting date so that the maximum population of thrips does not coincide with the

seedling stage. Earlier studies conducted on the insect pest were done in Zaria (Sub-humid zone of Nigeria) by Raheja (1973) which reported that population of thrips gradually built up and reached a peak 50 days after transplanting. Also Kisha (1977) found that few thrips were present on onion crop until mid-February when there was a sudden increase in numbers to peak levels during the first week of April, after which the numbers declined. There has been no study conducted on the seasonal abundance on the insect in this agro ecology (Dry Sub-humid) where bulk of the crop is produced. Therefore, this study was initiated with the following objectives:

(i) to study the incidence of thrips on un sprayed onion crop;

(ii) to assess changes in number of thrips on onion transplanted at different times of the growing season and

(iii) to identify the time of peak incidence and decline.

Therefore this paper reports the study conducted on the seasonal abundance of onion thrips, *Thrips tabaci* in Sokoto, Nigeria

2. Materials and methods

2.1 Experimental Area

Field experiments were conducted at Kwalkwalawa in Sokoto State, about 5 km from the main campus of Usmanu Danfodiyo University, Sokoto in the dry seasons of 2000/2001, 2001/2002 and 2002/2003 as indicated in the planting and transplanting dates below. The sites of the experiments remained the same in the three years. The area is located on latitude 13^0 01' and longitude $05^0$15'E, 300 m above sea level. Onion seeds of Ex-Gidan Kwano were raised at monthly interval in the nursery for 8 weeks before transplanting. The seedlings were transplanted by placing them in to holes, made with a sharp pointed stick at nearly the same depth they were in the nursery, except in P1 in 2001/2002, where the planting did not produce enough for transplanting. Poultry manure at the rate of 10 t/ha was applied and complemented by the application of N.P.K. (15:15:15) fertilizer at 2 and urea at 6 WAT. At 2 WAT, 300 kg of N. P. K. was applied (45 Kg N.P.K.) and later 97.8 kg/ha of urea (46 %N). The design was randomized complete block, replicated three times. The plot size was 2.5 m x 1.5 m containing 5 rows of 17 plants/ row, in a spacing of 30 cm between and 15 cm within row.

The planting and transplanting were as follows:

2001/2002 season	**2002/2003 season**
P_1 18/9/2001 (no transplanting)	17/9/2002; 12/11/2002
P_2 16/10/2001; 11/12/2001	15/10/2002; 10/12/2002
P_3 13/11/2001; 8/1/2002	12/11/2002; 7/1/2003
P_4 11/12/2001; 5/2/2002	10/12/2002; 4/2/2003
P_5 8/1/2002; 5/3/2002	7/1/2003; 4/3/2003

2.2 Sampling of Thrips from Onion Plants

Two plants were selected by systematic sampling from the 2nd and 4th rows at weekly intervals from each plot and placed in a labelled polythene bag. The height of each plant was measured from ground level to the tip of the tallest leaf. The choice of systematic sampling was to avoid sampling the same plant more than once to avoid sampling a plant twice, because onion has the ability to regenerate. It was observed that on the November and December transplants it was difficult to distinguish between excised and fresh (un-sampled) plants at 8-9 WAT and the only distinguishing feature was corrugation of leaves in the sampled plants. Onion plants were cut with a sharp knife close to the ground, placed in polythene bags before they were taken to the laboratory where they were placed in a deep freezer overnight to immobilize the insects.

2.3 Sampling of Thrips from Water Traps

Water traps were constructed from plastic containers16 cm outer and 13.5 cm inner diametre and 19.2 cm deep placed on metal plates welded to a metallic stand. The metallic stands were of sufficient length to be driven into the soil and pulled out from time to time for the trap to be adjusted to the height of the plants as the season progressed. The water traps were placed 2 metres apart in the field. This started when onion was transplanted and continued up to harvest.

Each trap was 3/4 filled with an aqueous solution made from 20 ml of liquid detergent and formalin, in a ratio of 1:3 in 4 litres of water. The liquid detergent was to reduce surface tension and formalin to act as a preservative (Adesiyun, 1977). The same concentration of the solution was maintained throughout the study period, as it was shown by Mayer (1961) that different concentration affects the number of insects caught. The contents of the traps were emptied into a sieve made of a fine clean cloth on weekly basis, soon after collection. The insects were collected into a vial containing 70 % ethanol before examination in the laboratory under a binocular microscope.

3. Results

Figure 1 shows that there was only one transplant in November in 2002/2003 season, as the equivalent crop did not survive at the nursery. Thrips started colonizing the crop in December in an insignificant number until end of January when the number rose to 20/plant and finally reached a peak at harvest without declining. The possible reason for the decline in thrips population between 3rd and 10th April was because the February transplanting was on the 5th February making it possible for insects to move to new plant. In the December transplant, the population was low in January and peaked in February in 2002/2003 and in 2001/2002; the peak was on 11th March (Figure 2). Figure 3 shows that there were variations in the abundance of thrips on onion in 2000/2001, 2001/2002 and 2002/2003 seasons. It reveals that in the three seasons, thrips were first noticed on the crop in the middle of February (11th) and the number remained low (< 5/plant) up to February 25 in 2000/2001 and 2001/2002 seasons. Thereafter, the number began to rise steeply. It peaked around March 18 in 2000/2001 season at 80 thrips/plant and about 280 thrips/plant in 2001/2002 season. It peaked earlier on March 11 at about 600 thrips /plant in 2002/2003.

In 2000/2001 and 2001/2002 seasons, the peak population of thrips occurred 6 weeks after first appearance on the crop; it took only five weeks in 2002/2003 season. The peak populations in 2000/2001, 2001/2002 and 2002/2003 seasons were 80 thrips/plant, 280 thrips/plant and 600 thrips/plant respectively. This progressive increase over three seasons may be attributed to population build-up on the piece of land over the years as these crops did not receive any spray made them very susceptible to attack.

In Figure 4 where onion plants were transplanted in February, there were two peaks in March and April in both years, though the population was slightly higher in 2001/2002 season. This could because there was a decline in January transplant, but the population rose again because already the first and second crops have been removed and only this and March crop might be producing new leaves. The population of thrips was high when onion was young and later declined (Figure 5) though there was some increase later, before finally declining at the time when the crop was ready for harvest. The possible reason for the differences in the two years in Figure 5 was November transplant provided enough breeding for subsequent crops, such as March transplant. Relative estimates of insects were obtained by the use of water traps, which were set up on a farmer's field from December to April to monitor flying thrips. Results (Figure 6) showed that catches were made from mid-December, but population of thrips remained low (less than 10 thrips/trap) until mid-March when population of more than 10 thrips/trap was recorded. The population continued to rise up to the third week of March and declined between 22 and 29 March but rose sharply to more than 70 thrips/trap by the first week of April. The experiment was terminated on April 7 because the crop was harvested two days to the sampling.

Yield of onion as presented in Figure 7 showed that between 30 and 50 t/ha was obtained when transplanting was done between months of November and December, but delaying till January results in less than 15 t/ha because by January the population of thrips in the month was over 50 thrips/plant in untreated plot at 7 WAT (Ibrahim and Adesiyun, in press)

4. Discussion

The low number of thrips recorded in 2002/2003 season compared to 2001/2002 in Figure 2,4 and 5 as against Figure 3 was probably due to early presence of the onion crop in November (Figure 1), which reached up to 180 thrips /plant when already three transplantings were on the field. This agrees with the findings of Kisha (1977) that in the first season, few thrips were present until mid-February when there was sudden increase in numbers to peak levels during the first week of April. However, the highest population of over 600 thrips/plant recorded in the January transplant indicates that the population was rising yearly because of continuous cropping. Adesiyun (1981; 1982) found that damage caused by shoot flies to sorghum planted early in the season was low and insignificant. He also maintained that the low population was on the scanty vegetation during the dry season. In this study, the population of thrips was low probably because many suitable hosts were unavailable and breeding sites inundated, but since this pest is polyphagous it could have survived on the wild plants. Reuda and Shelton (2003) reported that at the end of the season, however, thrips might not be able to survive in abundance because there is not sufficient green vegetation in the surrounding areas, as April and May being the driest months of the year. They added that from June-September heavy rains maintained thrips population at low levels in native vegetation where they were not treated, i.e. living on wild flora. This is evident in Figure 3 where the peak in 2000/2001season was less than 100 thrips/ plant, between 18th and 25th March, less than 300 thrips/plant in 2001/2002 season on the 11th March, 2002 and over 600 thrips/plant in 2002/2003 season on the 11th March, 2003. Reitz (2002) reported that populations of *Frankliniella occidentalis* Pergande did not reach to peak level

until the first week of May, but their population decreased over the next three weeks. There were variations in the peak populations, which ranged from 5 WAT in the March transplant, 9 or 10 WAT in the January transplant to 13 WAT in the November transplant. This disagrees with Raheja (1973) that the peak population of onion thrips was at 50 days after transplanting, probably because he used one planting.

Salguero-Navas *et al*. (1991) found that host plant phenology plays an important role in population dynamics, with younger plants being able to support greater densities than older plants, but this was found to be true with onions transplanted in February and March. Earlier transplantings made in November to December had peaks towards the end of the season. Similarly, Kannon and Mohammed (2001) observed that there was a steady increase of thrips population from February and March and a sharp decline in April in 1992/93 and 1993/94 growing seasons. Kisha (1977) reported that independent of transplanting date, thrips bred only between February and April and that high temperature and low humidity from April onwards were responsible for sudden population decline. In water traps where only adult thrips were recorded, the peak populations of more than 70 thrips/trap was recorded at the time of harvest was probably due to migration into the onion, which was harvested two days to the sampling date. Another possible reason was the movement of the thrips out of the onion at the time of harvest

5. Conclusion

The research has given the population dynamics of onion thrips in the three years (2000-2003), and has indicated that the November transplant had peak of onion thrips at the time of harvest and the crop has therefore escaped attack, yielding up to 50t/ha, but subsequent plantings such as December, January and February transplanting had their peaks in March. Also the trend of yield was found to be very similar in both years, where significantly higher yields were obtained in November and December transplants.

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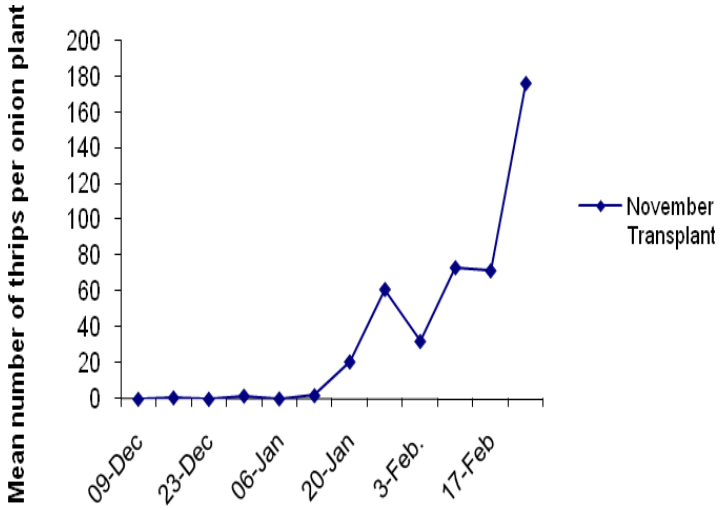

Fig: 1 Mean number of thrips per onion plant in 2002/2003 season

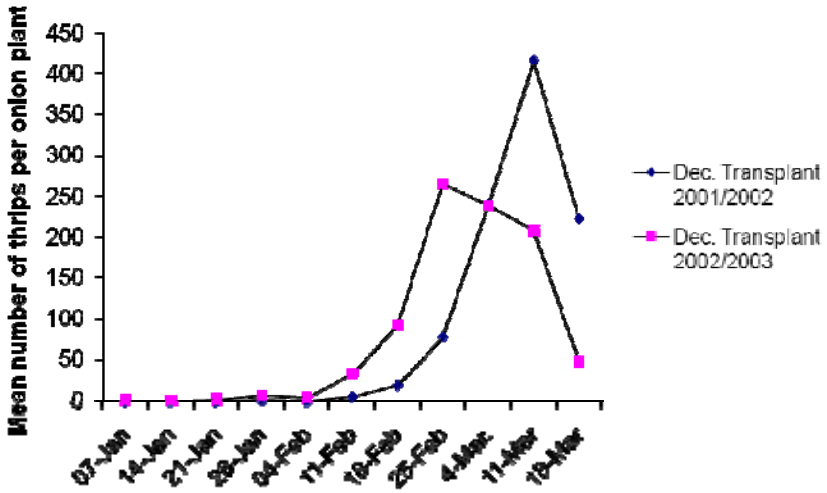

Fig 2: Mean number of thrips per onion plant 2001/2002 and 2002/2003 seasons

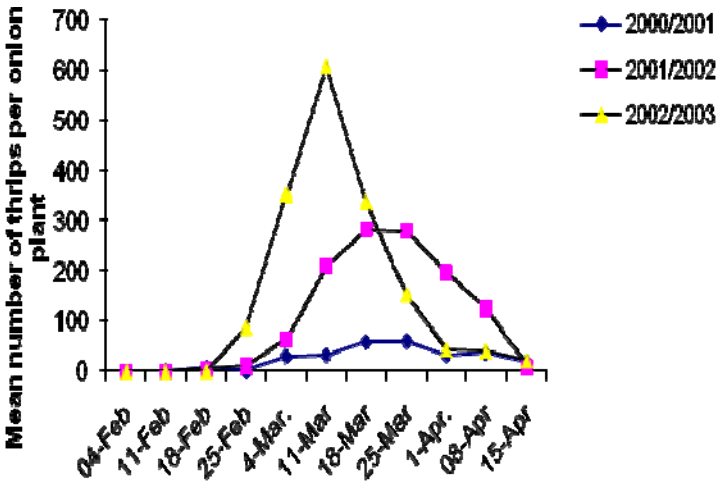

Fig 3: Mean number of thrips per onion plant in January transplant in 2000/2001, 2001/2002 and 2002/2003 seasons

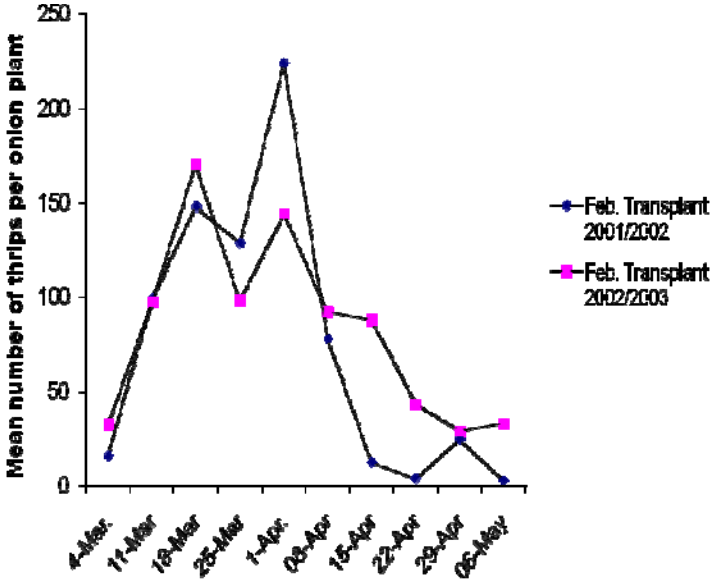

Fig 4: Mean number of thrips per onion plant in 2001/2002 and 2002/2003 seasons

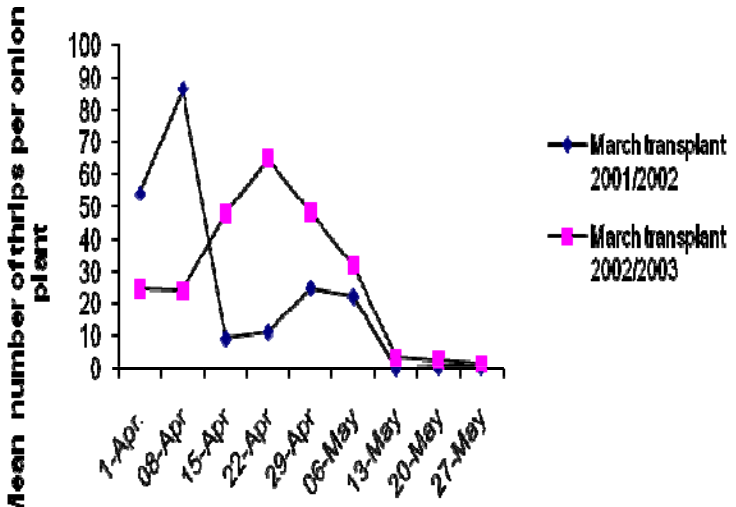

Fig 5: Mean number of thrips per onion plant in 2001/2002 and 2002/2003 seasons

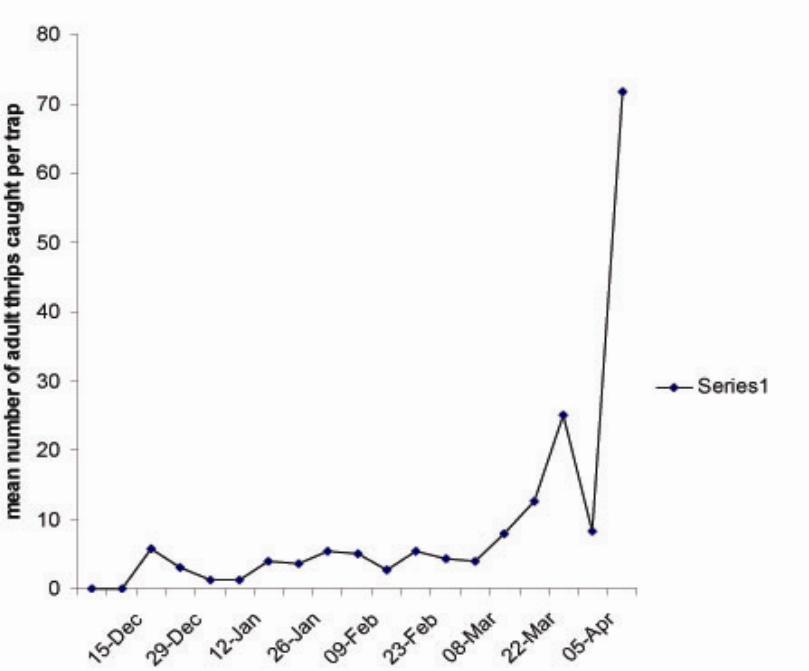

Fig 6: Weekly catches of adult thrips in water traps in 2003/2004 season

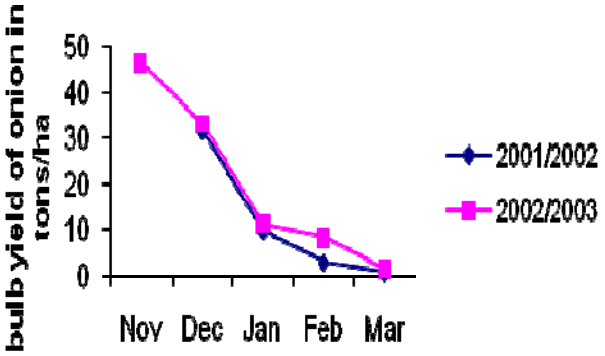

Fig 7: Onion bulb yield/ha in two seasons

Journal of Agricultural Science
http://www.ccsenet.org/jas
Vol. 2, No. 1
March 2010

Influence of Rhizobacterial and Agrobacterial Inoculation on Selected Physiological and Biochemical Changes of Banana Cultivar, Berangan (AAA) Plantlets

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This project is financed by Ministry of Science, Technology and Innovation (MOSTI), Malaysia (Sponsoring information)

Abstract

A series of experiments were carried out to observe the effects of rhizobacterial and agrobacterial inoculation, singly or combined on the total content, concentration and distribution of the biochemical components (total soluble protein, soluble nitrogen, proline, peroxidase activity, total soluble phenolic, nitrate reductase activity, nitrate, chlorophyll), physiological characteristics (percentages of growth, number of roots, fresh and dry weight of roots, maximum and total length of roots) and mineral contents (N, P, K, Ca and Mg) of *in vitro* banana plantlets, Berangan cultivar (AAA) using MS basal medium. The aims of this study are to determine the influence of various *rhizobacteria* sp. and *Agrobacteria* sp. inoculation, singly and combined on biochemical and physiological changes of the important banana plantlets in Malaysia, Berangan cultivar (AAA).Results from the inoculation study using MS basal medium were indicated that

inoculation with rhizobacteria (*Azospirillum brasilense* Sp7, *Bacillus sphaericus* UPMB10 and *Microbacterium oxydens* UPMB11) or agrobacteria (*Agrobacterium rhizogenes* strains, AR9402 and A4) showed positive response on growth of in *vitro* banana plantlets compared to uninoculation after one month of experiment. The inoculation treatments also increased the number of root, fresh and dry weight of roots and total length of root. In addition with inoculation, the total content or concentration of the respective biochemical activity as total soluble protein, peroxidase, nitrate reductase, proline, nitrate, soluble nitrogen, phenolic and chlorophyll of the host plants increased and varied according to the type of bacteria used. Inoculation with these bacterial also enhanced the accumulation of N and P in the banana plantlets. Co-inoculation with rhizobacteria (*Azospirillum brasilense* Sp7, *Bacillus sphaericus* UPMB10 and *Microbacterium* o*xydens* UPMB11) and agrobacteria (*Agrobacterium rhizogenes* strains AR9402 and A4) also showed similar response as in single inoculation; UPMB10+AR9402 treatment was the most effective treatment. The above finding provided evidence that *Azospirillum brasilense* Sp7, *Bacillus sphaericus* UPMB10, *Microbacterium* o*xydens* UPMB11, *Agrobacterium rhizogenes* strains AR9402 and A4, singly or combined are potentially effective in promoting growth of *in vitro* banana plantlets. Inoculation of rhizobacteria was showed beneficial to the banana plantlet in saline conditions through increment of growth and improvement in rooting system. Thus, these bacterial strains could be used as a bioenhancer for growth of *in vitro* banana plantlets.

Keywords: Rhizobacterial, Agrobacterial, Banana, Physiology, Biochemical

1. Introduction

Challenges faced by the banana industries from globalization are a result of lack of increased production and productivity, absence of economical scale of production, higher cost of inputs and low levels of technology used in the production system. Rhizobacteria that exert beneficial effects on plant growth and development are referred to as plant growth-promoting rhizobacteria (PGPR).

The use of PGPR to promote plant growth has increased in various parts of the world. PGPR can affect plant growth by producing and releasing secondary metabolites and facilitate the availability and uptake of certain nutrients from the root environment (Zahir *et al.*, 2003). A promising trend for increasing the efficiency of nitrogen fixing bacteria could be used naturally or through artificial mixtures of microorganisms (Okon and Labandera-Gonzalez, 1994). *Herbaspirillum seropedicae* is a nitrogen-fixing bacteria found in association with economically important plants (Bashan *et al.*, 2000).

Inoculation with *Azospirillum halopraeferens*, a mixture of two *Azospirillum brasilense* strains, a mixture of *Bacillus licheniformis* and *Phyllobacterium* sp. has significantly increased plant height and dry weight of oilseed (*Salicornia bigelovii*) (Bashan *et al.*, 2000). Co-inoculation is based on mixed culture inoculation, combinations of microorganisms that interact synergistically, or when rhizobacteria is functioning as a *'helper'* bacterium to enhance the performance of other beneficial microorganisms. Co-inoculation of a *Pseudomonas* sp. with *Mesorhizobium* strain (Ca181) has shown a significant increase in nodule weight and shoots biomass of *Vigna radiata*, when grown in sterilized condition (Sindhu *et al.*, 2002). The objectives of this study are to determine the influence of various *rhizobacteria* sp. and *Agrobacteria* sp. inoculation, singly and combined on biochemical and physiological changes of the important banana plantlets in Malaysia, Berangan cultivar (AAA).

2. Materials and methods

2.1 Plant materials

Banana plantlets cv. Berangan (AAA) established in a standard MS solid medium (Murashige and Skoog, 1962). One-month-old plantlets were cultured in 30 mL modified MS liquid medium at pH 5.7 using 100 mL Erlenmeyer flasks. The cultures were incubated on an orbital shaker at 80rpm and were exposed to continuous fluorescent light at $27^{o}C \pm 1^{o}C$ for a month. The one-month duration was sufficient for plantlets to absorb all nutrients that were available in the media. The 30 mL MS liquid medium was replenish with fresh medium at two week intervals.

2.2 Bacterial cultures

Rhizobacterial and agrobacterial cultures were used in this experiment. The treatments were: inoculated, singly or combined with 1 mL of different species of rhizobacteria and agrobacteria into the MS liquid media at $1x10^{7}$-$1x10^{8}$ cfu/mL (OD_{600nm}) concentrations.

Experiments were divided into three sequential experiments; inoculated singly with rhizobacteria (**Experiment A**), or agrobacteria (**Experiment B**) and combined (rhizobacteria and Agrobacteria) (**Experiment C**). The medium were inoculated with different species of rhizobacterial and agrobacterial, singly or combined at $1x10^{7}$-$1x10^{8}$ cfu/mL (OD_{600nm}) concentrations.

Experiment A: The three species of rhizobacterial used were: *Azospirillum brasilense* Sp7, *Bacillus sphaericus* UPMB10 and *Microbacterium oxydens* UPMB11. The non-inoculated treatment was used as a control.

Experiment B: The four different strains of agrobacterial used were: *Agrobacterium rhizogenes* strains AR9402, A4, 16758 and 14356. The non-inoculated treatment was used as a control.

Experiment C: Co-inoculation (mixed cultures) treatment of the rhizobacteria and agrobacteria were carried out using *Azospirillum brasilense* Sp7, *Bacillus sphaericus* UPMB10 or *Microbacterium oxydens* UPMB11 mixed with *Agrobacterium rhizogenes* strains AR9402 or A4. A single inoculated treatment using *Bacillus sphaericus* UPMB10 and non-inoculated treatment were used as controls.

Plantlets that have been inoculated with the respective bacterial treatments were placed on orbital shaker (80 rpm) at 27±1^{o}C under continuous florescent light. Physiological parameters recorded include plant growth, number, fresh and dry weight and maximum and total length of roots.

Similarly, biochemical changes such as total soluble protein content (Bradford, 1976), soluble nitrogen Speis (1957), proline (Bates, 1973), peroxidase activity, total soluble phenolic content, nitrate reductase activity (Andrew *et al.*, 1992), nitrate content, and chlorophyll content (Harbone, 1973) after one month of culture. To determine the percentage of N, P and K in plantlets, Digesdahl Digestion Apparatus Methods were used. The whole plantlets were dried at 60^{o}C for 2 or 3 days and their weights were recorded. A 0.2 g of samples was grinded. The samples (dry plantlets) were put into a 100 ml digestion flask. A 5 ml of concentrated sulphuric acid (H_2SO_4) was added into the digestion flask and swirled to get an even mixture. Meanwhile, the digesdahl apparatus were set at 44^{o}C. The samples were heated until white smoke disappeared or after 5 to 10 minutes. A 5 ml of hydrogen peroxide (H_2O_2) was added and digestion continued until the boiling ceased and clear samples were obtained indicative of complete digestion. The samples were removed from the digestion apparatus to cool and the volumes were adjusted to a final 100 ml with distilled water. The solution was then filtered using Whatman Paper No. 42 and collected in plastic vials. The N and P concentration were determined by using auto analyser apparatus. The samples were taken directly from the filtrate. For K determination, the filtrate needs to be diluted to 25 times. The samples for K concentration were determined using the flame photometer. All the nutrients determination was carried out in the Analytical Laboratory, Department of Land Management, and Faculty of Agriculture UPM.

2.3 Statistical analysis

The experimental were set up with 10 replicates and repeated twice. The result were compared by ANOVA and tested by Duncan's multiple range test to find the differences between treatment means at the 5% (0.05) significant level. Data were analyzed using the general Statistical Analysis system (SAS).

3. Results and discussion

3.1 Growth and root biomass

In experiment A, single inoculation with rhizobacterial species experienced an increase in growth of the plantlets, number, fresh and dry weight, and total length of roots of *in vitro* banana plantlets (Figure 1). Results showed that single inoculation with UPMB10 had the highest increase in growth of plantlet at 172% (control is 119 %) **(**Figure 1a;2). Inoculation with UPMB11 also showed an increase up to 35.3% but Sp7 did not produce a significant increase (4.2%) when compared to the control. The results showed that rhizobacterial species UPMB10 and UPMB11 have the ability to increase plant growth under *in vitro* conditions after one month of treatment.

Figure 1

Figure 2

Inoculation with all three species of rhizobacteria increased the root number of banana plantlets compared to the control (Figure 1 a-b). Inoculation with Sp7 gave the highest increment at 7, followed by UPMB11 and UPMB10 treatments, both gave similar increment, 5. The root fresh weight also showed an increase when inoculated with Sp7 and UPMB11 at 0.78 g and 0.79 g, respectively (Figure 1 a-c**).** Inoculation with UPMB10 showed no change in weight compared to the control. Inoculation with UPMB11 showed an increase in root dry weight at 40.3 mg while the other treatments showed no change in weight compared to the control (Figure 1 a-d). The maximum root length showed a significant ($P<0.05$) increase only in UPMB10 treatment at 29.5 cm while the others showed no significant change (Figure a-e). The total root lengths showed an increase only in UPMB11 and UPMB10 treatments at 47 and 46.3 cm, respectively, compared to control while Sp7 produced no significant ($P<0.05$) change (Figure 2).

In Experiment B, inoculation with agrobacterial on *in vitro* banana plantlets showed inherent effects in enhancing growth of the host plant. The growth of plantlets and root biomass as number, fresh and dry weight of roots, maximum and total lengths of root are shown in Figure 3. Generally, inoculation with strains of AR9402 and A4 showed increased growth of plantlets (211-181%) (Figure 3) compared to the control. In addition plantlets treated with agrobacterial strains 15834 and 8189 exhibited growth within a range 43-61%, significant lower than the control (Figure 4). Sindhu *et al.* (2002) reported that production of toxic metabolites with an inhibitory effect on growth of roots by non-fluorescent *Pseudomonads*.

Figure 3

Figure 4

The experiment has shown that inoculation of agrobacteria especially from strain AR9402 and A4 have the potential to stimulate growth and root biomass of the host plant. Inoculation of *Agrobacterium* strains AR9402 and A4 have shown inherent effect in stimulating growth and rooting system of the *in vitro* banana plantlets. It is well established that *Agrobacterium* species play an important part in promoting rooting system of plants. Based on the results, it is interesting to highlight that the association of agrobacteria (strain AR9402 and A4) and plantlets have successfully enhanced growth and rooting system of the host plant. According to Freitas *et al.* (1997), the most common bacteria associated with roots of field crops were bacilli (34%) and pseudomonads (17%), with the most abundant being *B.brevis, B. licheniformis, B. megaterium* and *B. sphaericus*.

In Experiment C, the combined treatments of rhizobacteria and agrobacteria were tested in association with *in vitro* banana plantlets. The data of six combined inoculation treatments with rhizobacterial and agrobacterial on plant growth, number, fresh and dry weight, maximum and total length of roots are shown in Table 1. The plant growth highest in UPMB10+A4 (685.5%) treatment, followed by UPMB11+AR (673%), UPMB11+A4 (641%) and UPMB10+A4 (435.5) treatments. The Sp7+AR (409%) treatment showing no change compared to control (Table 1).

Table 1

These results suggested that rhizobacteria (UPMB10 and UPMB11) acted synergistically with agrobacteria (AR940 and A4) and were effective in promoting growth of the plantlets. Thus, these rhizobacteria could be exploited as co-inoculants with agrobacteria for better plant growth. Similar results were obtained by Shindu *et al.* (2002) with co-inoclulation of *Pseudomonas* strain and *Mesorhizobium* which stimulated nodule fresh weight and plant dry weight. Thus, combination of rhizobacteria and agrobacteria should be evaluated since they may have a potential as future inoculants

3.2 Total soluble protein

Total soluble protein in leaves and roots increased after inoculation with Sp7 and UPMB10 with no change observed for UPMB11 treatment, compared to control (Figure 5). The total soluble proteins in leaves for both Sp7 and UPMB10 are much higher than in roots with those for leaves at 15.0 mg/gfw and 19.0 mg/gfw and for roots at 14.0 mg/gfw and 15.8 mg/gfw, respectively.

Total soluble protein content in leaves and roots were also markedly increased by inoculation with agrobacterial strains AR9402 and A4 but showed a reduction with strains 15834 and 8189. The total soluble proteins in leaves for both agrobacteria strains AR9402 and A4 are much higher than in roots with those for leaves at 24 mg/gfw and 25 mg/gfw and for roots at 16.6 mg/gfw and 18.9 mg/gfw, respectively. It was observed that the soluble protein increased only in treatment inoculated with agrobacteria strains AR9402 and A4, which showed similar trend in growth and root biomass of plantlets as shown in Figure 5b.

The highest leaf protein content was obtained from plantlets that were co-inoculated with different species of rhizobacterial and agrobacterial. It was UPMB10+AR treatment (20 mg/gfw) followed by UPMB11+AR (19.8 mg/gfw), UPMB11+A4 (18.96 mg/gfw) and UPMB10+A4 (18 mg/gfw) treatments (Figure 5c). Similar trends were observed in root protein of plantlets whereby, UPMB10+AR treatment showed the highest in root protein at 19.5 mg/gfw compared to other treatments.

Figure 5

The results showed that the combined inoculation of rhizobacteria and agrobacteria affected the amount of total soluble protein of plantlets. Combined inoculation with UPMB10 and agrobacterial strains AR9204 produced superior result in protein content compared to other treatments. The increase of total protein content of plantlets could be related to the enhancement of root growth and stimulation on nutrient uptake.

3.3 Soluble nitrogen

Inoculation with three species of rhizobacterial into the media did not produce a significant increase in leaf soluble nitrogen content of plantlets when compared to the control (Figure 6). Inoculation with UPMB10 showed the highest increase in root soluble nitrogen content which increased up to 23.2%, followed by Sp7 and UPMB11 treatments at 21.5% and 20.3%, respectively (Figure 6a). The results showed that all species of rhizobacterial have the ability to increase soluble nitrogen content of banana plantlets only roots, not in leaves.

Figure 6

Inoculation with agrobacterial strains A4 showed the highest soluble nitrogen content of *in vitro* banana plantlets in both leaves and roots at 389 μg/gfw and 235 μg/gfw, respectively. Inoculation with agrobacterial srains AR9402 also show significant increase in leaves and roots soluble nitrogen content of plantlets at 345 μg/gfw and 213 μg/gfw

compared to the control. But those with treatment 15834 (leaves: 150.0 μg/g fw, root: 98.6 μg/g fw) and 8189 (leaves: 180.0 μg/g fw, roots: 99.5 μg/g fw) showed significantly lower than the control (Figure 6b). These phenomena indicated the ability of the inocula tested, *Agrobacterium rhizogenus* strain AR9402 and A4 to increase the content of soluble nitrogen of the *in vitro* banana plantlets

An increase was also observed in treatments of UPMB11+AR, UPMB11+A4 and UPMB10+A4 (Figure 6c). These results indicated that combined inoculation treatments directly affect the soluble nitrogen content of plantlets, as physiological effects (percentages of growth, number of roots, fresh and dry weight of roots, maximum and total length of roots). The mechanism of growth promotion by co-inoculation is not well understood; however a wide range of possibilities have been postulated including an increase in insoluble nutrient and subsequent enhancement in uptake capacity by plant which eventually will stimulate plant growth (Bashan and Holguin, 1995).

3.4 Proline

Inoculation with rhizobacteria showed dramatic decrease in proline content of roots for all species compared to control (Figure 7). The descending order of effect of rhizobacterial inoculation on root proline content: UPMB11>Sp7>UPMB10 with values of 58 ug/gfw, 45.6 μg/gfw and 34.2 μg/gfw compared to control at 126.7 μg/gfw **(**Figure 7a). There is an increase in proline content of leaves for Sp7 treatment at 49.7 μg/gfw compared to control at 35.0 μg/gfw.

Figure 7

Plants inoculated with agrobacterial strains 15834 and 8189 showed higher proline content compared to the control in both leaves and roots within a range 77-85 μg/gfw and 178-241 μg/gfw, respectively (Figure 7b). High accumulation of proline content in the treatment inoculated with agrobacterial strains 15834 and 8189 could be related indirectly to the inoculation process retarding root growth and development of the plantlets. Similar reports have been shown by Wang *et al.* (1999) that a decrease in growth rate was accompanied by an increase in proline level. Proline is considered to be involved in osmotic adjustment (Wang *et al.*, 1999). The beneficial role of proline in plant stress tolerance demonstrated that proline can increase the tolerance of plant to abiotic stress (Saleena *et al.*, 2002).

Combined inoculation of rhizobacteria (Sp7) and agrobacteria (strains AR9402 or A4) showed a higher level of proline content in both leaves and roots compared to other treatments. The distribution of proline accumulation was more in the roots rather than leaves since the roots are in contact with the medium and inocula. Proline content of leaves of the plantlets treated with Sp7+A4 and Sp7+AR was higher compared to other treatments at 78.4 μg/mg protein and 91.4 μg/mg protein, respectively. For other treated plantlets, the leaf proline content was within a range 31.0-44.0 μg/mg protein with the control at 39.0 μg/mg protein. Similar result were observed in roots of plantlets: Sp7+A4 treatment showed the highest proline content at 181.8 ug/mg protein followed by Sp7+AR9402 treatment at 167.4 μg/mg protein and other treatments within a range 29.0-65.0 μg/mg protein and control at 151.8 μg/mg protein (Figure 7c). Based on the result on plant growth, combined inoculation of Sp7 with AR9402 or A4 showed inhibited plant growth (Table 1).

3.5 Peroxidase activity

Peroxidase activity after inoculation with UPMB10 showed an increase to 1896 U/mg protein in leaves and to 3897 U/mg protein in roots compared to the control (Figure 8). Treatment with other species did not show significant change in peroxidase activity compared to the control in both leaves and roots. Plant peroxidase can reinforce the cell wall through the deposition of several cell wall components such as lignin, suberin and extensin (Lagrimini, 1996).

Figure 8

Leaves of plantlets inoculated with agrobacterial strains 8189 showed highest peroxidase activity at 5136 U/mg protein, followed by plants inoculated with agrobacterial strains 15834, AR9402 and A4 at 2400 U/mg protein, 3001 U/mg protein and 1900 U/mg protein, respectively compared to the control at 2500 U/mg protein. Similarly in root, the peroxidase activities were higher in the presence of agrobacteria in medium to within a range 5647-9230 U/mg protein with the control (without agrobacteria) at 3463 U/mg protein (Figure 8b). The precise function of peroxidase in plant growth, development and stress tolerance remained unclear though evidence indicated that peroxidase is involved in host defense and stress induced lignification (Lagrimini *et al.*, 1996).

The data presented in Figure 8c show the combined inoculation of rhizbacteria and agrobacteria on peroxidase activity of plantlets. Highest peroxidase activity was observed in UPMB10+ AR9204 treatment in both leaves and roots at 3514 U/mg protein and 9423 U/mg protein, respectively. Combined inoculation of rhizobacteria Sp7 with agrobacterial strains AR9204 or A4 showed a decrease in peroxidase activity compared to uninoculated or single inoculation of banana plantlets.

3.6 Phenolic compound

Inoculation with rhizobacteria showed an increase in phenolic content of roots and leaves of plantlets compared to the control (Figure 9). In roots, UPMB10 treatment showed the highest in total soluble phenolic at 63.07 μg/gfw followed

by the UPMB11 and SP7 treatments at 61.5 µg/gfw and 56.5 µg/gfw, respectively compared to control which was only at 55.25 µg/gfw (Figure 9a). Similar results were obtained in leaves of plantlet, which UPMB10 gave at 27 mg/gfw, UPMB11; 15.7mg/gfw and SP7; 11.2 mg/gfw and control was only at 10 mg/gfw.

Figure 9

The total soluble phenolic in roots for both treatment, AR9402 and A4 are much higher than in leaves at 76.4 mg/gfw and 84.9 mg/gfw foor roots and at 13.0 mg/gfw and 15.7 mg/gfw for leaves, respectively (Figure 9b). This result clearly demonstrated that both agrobacteria strains AR9402 and A4 could induce total phenolic compound especially in roots in association with banana plantlets under tissue culture condition. Report has shown that growth and accumulation of phenolic compounds are generally inversely related by altering the mineral uptake, water relation, photosynthesis, carbon flow, and phytohormone activity (Iosipenko and Ignatov, 1995).

3.7 Nitrate reductase activity (NRA)

Figure 10 shows NR activity in both leaves and roots of plantlets inoculated with rhizobacteria. Generally, there was an increase in NR activity for all treatments. The UPMB10 treatment showed the highest increment of NR activity in root at 0.2 U/mg protein. In leaves, the highest activity was showed by Sp7 treatment at 0.01 U/mg proteins. The descending orders of effectiveness of rhizobacteria were: UPMB10>UPMB11>Sp7 for leaves and UPMB10> Sp7>UPMB11 for roots. This study indicated that NR activity in leaves and root of plantlets increased when plantlets were grown in a media containing rhizobacteria.

Figure 10

In the subsequent analysis, the range of NR activity of plantlets inoculated with agrobacteria showed higher activities than those uninoculated. In roots, inoculation process showed an increase in NR activity of plantlets. The inoculation treatment showed a higher NR activity plantlets compared to control with a range 0.05-0.24 U/mg of protein (control at 0.01 U/mg protein). The highest was observed in AR9402 treatment at 0.24 U/mg protein. However, in leaves the NR activity was higher only in AR9402 and A4 treatments at 0.01 U/mg protein and 0.02 U/mg protein, respectively (Figure 10b). Nitrate reductase is a substrate - inducible enzyme and is thought to be the most limiting step in N assimilation. For this reason, NR activity could be selected for yields and N assimilation potential (Li and Oaks, 1993). The NO_3^- reduction is controlled primarily by the rate of NO_3^- uptake, rather than by alterations in NR activity or limitations in reducing power (Wilkinson and Crawford, 1993).

Figure 10c shows the effect of combined inoculation of rhizobacteria and agrobacteria on NR activity in leaves and root of the banana plantlets. Leaves NR activity was the highest in UPMB10+AR treatment at 0.25 U/mg protein and followed by treatment of UPMB11+AR (0.20 U/mg protein), UPMB11+A4 (0.13 U/mg protein), UPMB 10+A4 (0.12 U/mg protein) and Sp7+AR (0.12 U/mg protein).

3.8 Nitrate

Results showed that the nitrate content in leaves was much higher than that of roots (Figure 11). Inoculation of rhizobacteria showed nitrate is higher compared to uninoculated. The treatment that consists of rhizobacteria showed a higher content of nitrate compared to uninoculated in all parts of the plantlets. Results from Figure 11a shows the increase in the nitrate content of leaves in all the treatments of rhizobacteria species compared to control with UPMB11 giving the highest increment at 38.7 mg/gfw followed by UPMB10 and Sp7 treatments at 36.8 mg/gfw and 33.7 mg/gfw, respectively. Similarly in root, which UPMB10 shows the highest increment in nitrate content at 108.7 mg/gfw followed by UPMB11 and Sp7 treatments at 80.6 mg/gfw and 71.0 mg/gfw. The descending order of effectiveness of rhizobacteria was: UPMB11>UPMB10>Sp7 for leaves and UPMB10 >UPMB1> Sp7 for roots.

Figure 11

Nitrate accumulation for respective plantlets was influenced by different inoculation treatments. The highest results in nitrate content of plantlets were shown in treatment of AR9402 followed by A4 treatment in both leaves and root. The results shown were within the range of 39.0 – 42.0 mg/gfw in leaves and 51.0-54.0 mg/gfw in root (Figure 11b). The nitrate content in leaves and roots increased in combined treatments containing UPMB10 or UPMB11 combined either with agrobacteria strains AR or A4. The highest nitrate content in leaves and root was in UPMB11+AR (69.8 mg/gfw) and UPMB11+A4 (126.3 mg/gdw) treatments, respectively. The data presented in Figure 4.8-c reveal that combined inoculation of rhizobacteria and agrobacteria showed positive effect on quantities of nitrate content in leaves and root of banana plantlets especially both agrobacteria, strains AR9402 or A4 combined with UPMB10 or UPMB11.

Results showed that nitrate content was higher in combined treatments when compared to the control or single inoculation especially in root. The increase in nitrate content of banana plantlets is probably due to an increase in NR activity in the plantlets. The promotion of plant growth by PGPR for most parts of the plants has provided the plant with a compound that is synthesized by the bacterium to facilitate the uptake of certain nutrient from the environment (Glick, 1995).

3.9 Chlorophyll

Results from Figure 12 showed the total chlorophyll content of plantlets inoculated with rhizobacteria was higher compared to the control. UPMB10 treatment giving the highest of chlorophyll content at 7.5 mg/gfw followed by UPMB11 and Sp7 treatments at 6.4 mg/gfw and 5.2 mg/gfw, respectively. There was a positive response of inoculation in promoting chlorophyll content of plantlets

Figure 12

Total chlorophyll content in leaves was increased after inoculated with agrobacteria strains AR94027 and A4 (Figure 12). However, the total chlorophyll was reduced in plantlet inoculated by agrobacteria strains15834 and 8189. The total chlorophyll content in leaves for both treatment of AR9402 and A4 was much higher than the control at 6.0 mg/gfw and 5.9 mg/gfw, respectively. The chlorophyll content of plantlets inoculated with strains 15843 and 8198 shows much lower only at 0.23 mg/gfw and 0.46 mg/gfw compared to control at 4.56 mg/gfw (Figure 12b). Leaves chlorophyll content of plantlets in the combined inoculated of rhizobacteria and agrobacteria was more effective compared to the single inoculation. The UPMB10+AR9402 treatment showed the highest chlorophyll content at 6.93 mg/gfw followed by UPMB10+A4 treatment at 6.925 mg/gfw and single inoculation (UPMB10 treatment) showed only at 3.66 mg/gfw (Figure 12c).

According to Quilici and Medina (1998), photosynthetic and chlorophyll content increased linearly with leaf N content in both *Crotalaria* and *Verbasina*. De Veau *et al.* (1990) have reported that the mean milligram of chlorophyll content per square decimeter of *Bradyrhizobium* inoculated soybean (*Glycine* max) test leaves was about 50% lower than the other group's leaves of control. The inoculated host plants utilized their chlorophyll more efficiently for photosynthetic CO_2 uptake than control plants.

3.10 N, P, K, Ca and Mg content

Effects of rhizobacteria inoculation on nutrient uptake; Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca) and Magnesium (Mg) content of plantlet were analyzed and presented in Figure 13. The nitrogen level in plantlets inoculated with UPMB10, UPMB11 and Sp7 showed significant increase compared to those uninoculated (control) with an increment at 5.3 mg/gdw, 5.1 mg/gdw and 5.13 mg/gdw, respectively.

Potassium content in the plantlets inoculated with UPMB10 was highest at 5.6 mg/gdw. However treatment with Sp7 and UPMB11 showed significant lower than the control at 3.5 mg/gdw and 3.6 mg/gdw, respectively. Treatments with all species of rhizobacterial did not produce a significant change in Ca content of the plantlets compared to the control. According to Glick (1995), *Azospirillum* may increase the solubilization of minerals such as phosphorus and metal in the forms that are more readily available for plant growth. Increased mineral have been attributed to the increase in uptake to a general increase in the volume of the root system and not to any specific enhancement of the normal ion uptake mechanism (Murthy and Ladha, 1988). The plants may take up N more efficiently from the limited supply in the medium, resulting in lower requirement of N fertilizer to attain a certain yield. Supporting evidence for increased mineral uptake by inoculated roots is provided by an enhancement in proton efflux activity of wheat root inoculated with PGPR (Bashan *et al.*, 1990). It is well known that proton efflux activity is directly related to the balance of ions in plant roots.

Figure 13

The effect of agrobacteria inoculation on nutrient uptake of plantlets such as N, P, K, Ca and Mg contents are shown in Figure 13b. Nitrogen levels in plantlets showed a significant increase after inoculation with agrobacterial strains AR9402 and A4 at 7.8 mg/gdw and 7.9 mg/gdw respectively. But those treatments with 15834 (4.1 mg/gdw) and 8189 (5.3 mg/gdw) did not show a significant change compared to the control. Similarly was obtained in P content, it was strongly affected in plants inoculated with agrobacterial strains AR9402 and A4. K content of plantlet was the highest in treatment inoculated with strain AR9402 (8.09 mg/gdw) followed by A4 treatment (6.45mg/gdw). Calcium contents in plantlets inoculated with agrobacterial strains AR9402 and A4 increased up to 0.5 mg/gdw and 0.65 mg/gdw, respectively, compared to the control at 0.15mg/gdw.

The effect of combined inoculation on essential nutrient distribution in leaves and roots of banana plantlets is shown in Table 2. There was an increase in N, P, and K uptake in UPMB10 and UPMB11 treatments either combined with agrobacterial strains AR or A4 when compared to the single inoculation or the control. The combined inoculation of UPMB10 with agrobacterial strains AR9402 or A4 promoted the nutrient uptake (N, P, K, and Mg) of the host plant compared to single inoculation. The result showed that treatment with UPMB10+AR gave the highest N (6.71 mg/gdw), P (0.821 mg/gdw) and Mg (0.32 mg/gdw) uptake compared to other treatments.

Table 2

4. Conclusion

The experiments indicate that single inoculation with *Azospirillum brasilense* Sp7, *Bacillus sphaericus* UPMB10, *Microbacterium* o*xydens* UPMB11, *Agrobacteria rhizogenes* (strains AR9402 and A4) can enhance growth of *in vitro* banana plantlets. These bacterial strains become important to devise strategies to improve growth in tissue culture system through positive response on physiological characteristics as percentages of growth, number of roots, fresh and dry weight and total length of roots. At the same time, with inoculation the total content or the concentration of the respective biochemical activities, such as total soluble protein, peroxidase, nitrate reductase, proline, nitrate, soluble nitrogen, phenolic and chlorophyll of the host plants have increased but varied according to the type of bacteria used. The inoculation treatment also caused the increase in total N and P contents of the host plants. The combined inoculation treatment with rhizobacteria (strain UPMB10 or UPMB11) and agrobacteria (AR9402 or A4) enhanced plant growth when compared to the single inoculation. The combined inoculation has advantages over the single inoculation especially in producing higher biochemical activities of the plantlets. The combined inoculation treatment also enhanced nutrient uptake, especially N and P, compared to the single inoculation and therefore, can be considered as a bioenhancer for *in vitro* system. The results of the study clearly showed that combined inoculation of rhizobacteria and agrobacteria is a promising technique to enhance the growth of *in vitro* banana plantlets.

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Table 1. Effect of plant growth and root biomass of in vitro banana plantlets after one month culture in MS liquid medium and co-inoculated with rhizobacteria and agrobacteria. Values are means of ten replicates with ± SD (P=0.05, n=10).

Treatment	Percentage of growth (%)	Number of roots	Fresh weight of roots (g)	Dry weight of roots (mg)	Max. length of roots(cm)	Total length of roots (cm)
Control	364.5±35.00	4.6±0.50	0.7±0.07	32.00±5.60	24.0±2.30	30.0±6.50
UPMB10	402.9±26.00	6.5±0.80	0.6±0.10	43.4±4.50	30.1±1.50	45.8±2.80
UPMB10+ A4	435.5±54.00	9.5±0.40	0.6±0.03	41.6±6.00	21.0±4.10	46.4±11.0
UPMB10+ AR	685.5±26.00	10.2±1.50	1.1±0.11	74.5±15.00	35.6±1.10	76.0±2.10
UPMB11+ A4	641.0±54.00	5.0±1.10	0.9±0.04	38.7±11.00	26.7±1.20	54.0±8.70
UPMB11+ AR	672.0±78.00	7.5±0.50	0.9±0.09	70.4±5.40	27.1±2.50	78.9±6.50
SP7+A4	323.0±25.00	8.5±0.20	0.3±0.09	25.4±6.30	11.0±4.60	23.6±12.0
SP7+AR	409.2±50.00	6.5±1.2	0.6±0.14	74.2±5.00	25.1±1.50	69.6±6.60

Table 2. Nutrients content (mg/gdw) of in vitro banana plantlets after one month culture in MS liquid medium co-inoculated with rhizobacteria and agrobacteria. Values are means of ten replicates with ± SD (P=0.05, n=10).

	N (mg/gDW)	P (mg/gDW)	K (mg/gDW)	Ca (mg/gDW)	Mg (mg/gDW)
Control	4.50±0.23	0.08±0.05	6.78±0.40	0.19±0.10	0.27±0.02
UPMB10	4.52±0.45	0.62±0.02	5.14±0.35	0.89±0.08	0.26±0.01
UPMB10+A4	6.07±0.11	0.80±0.09	7.21±0.23	0.79±0.11	0.31±0.02
UPMB10+AR	6.71±0.56	0.82±0.05	6.16±0.46	0.81±0.07	0.32±0.02
UPMB11+A4	5.10±1.10	0.64±0.02	5.25±0.66	0.84±0.10	0.29±0.01
UPMB 11+A4	6.70±0.44	0.57±0.04	6.06±0.45	0.81±0.03	0.29±0.05
SP7+A4	4.50±0.24	0.52±0.05	5.01±0.13	0.67±0.09	0.14±0.05
SP7+AR	4.51±0.34	0.49±0.07	4.12±0.45	0.68±0.20	0.19±0.01

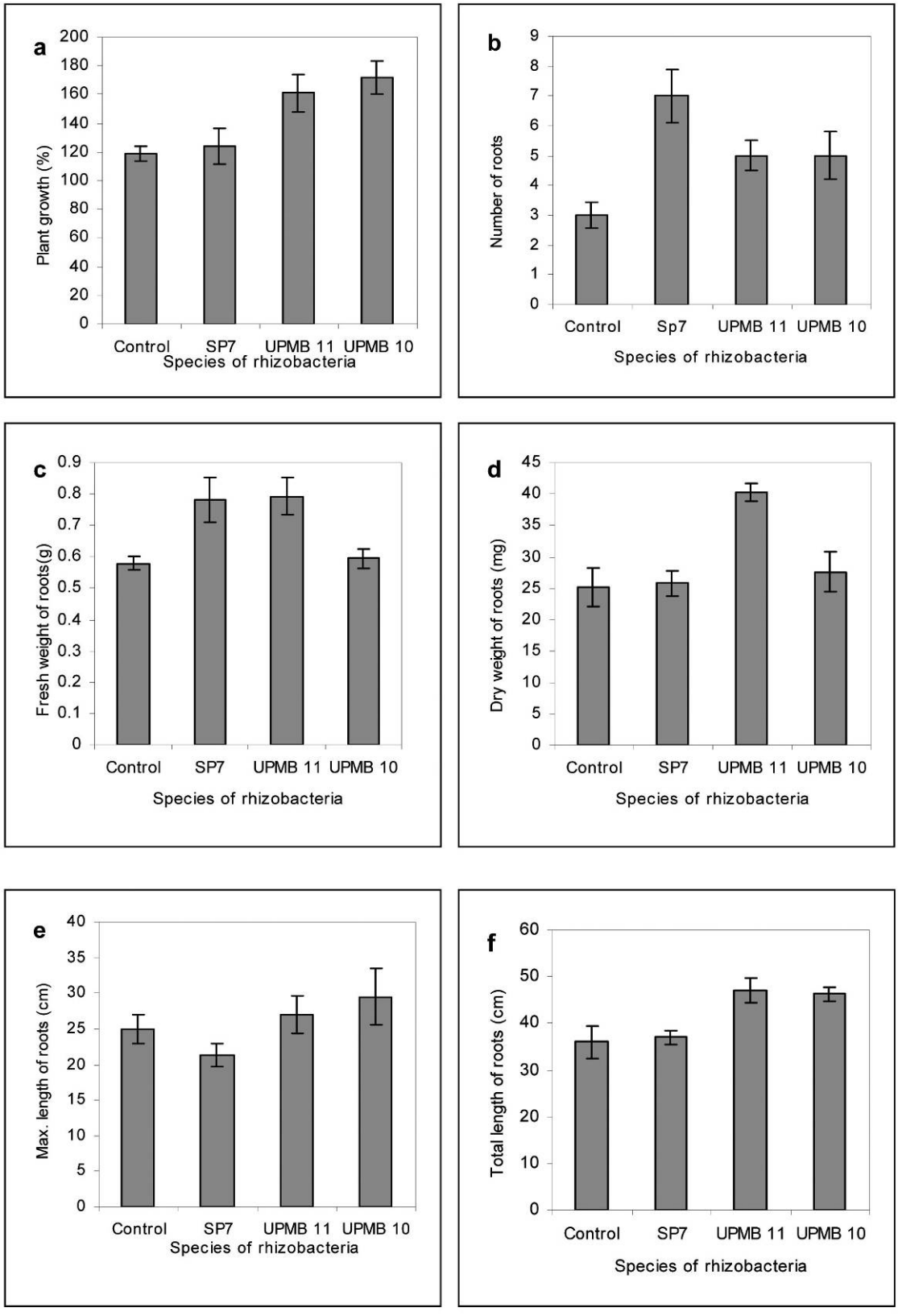


Figure 1. Growth of *in vitro* banana plantlets and root biomass after one month culture in MS liquid medium and inoculated with rhizobacterial species a (percentage of growth), b (number of roots), c (fresh weight of roots), d (dry weight of roots), and e (maximum length of roots) and f (total length of roots).Bars show means SD of ten replications (P=0.05, n=10).

Figure 2. Growth of in vitro banana plantlets after one month culture in MS liquid medium inoculated with rhizobacterial: A (control), B (Sp7), C (UPMB11) and D (UPMB10). Bar represents 10mm.

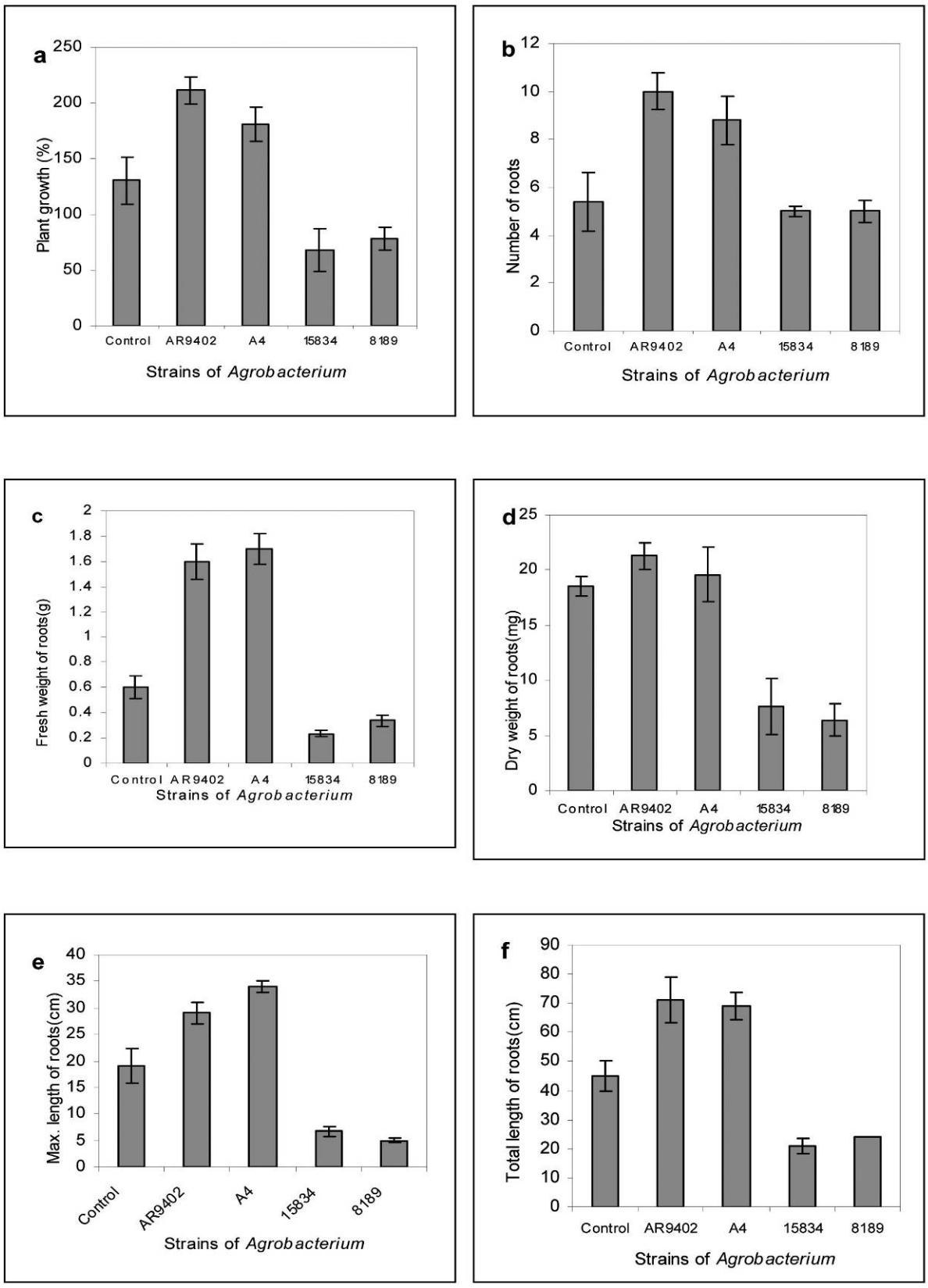


Figure 3. Growth and root biomass of in vitro banana plantlets after one month culture in MS liquid medium inoculated with strains of Agrobacterium: a (percentage of growth), b (number of roots), c (fresh weight of roots), d (dry weight of roots), and e (maximum length of roots) and f (total length of roots). Bars show means ± SD of ten replications (P=0.05, n=10).

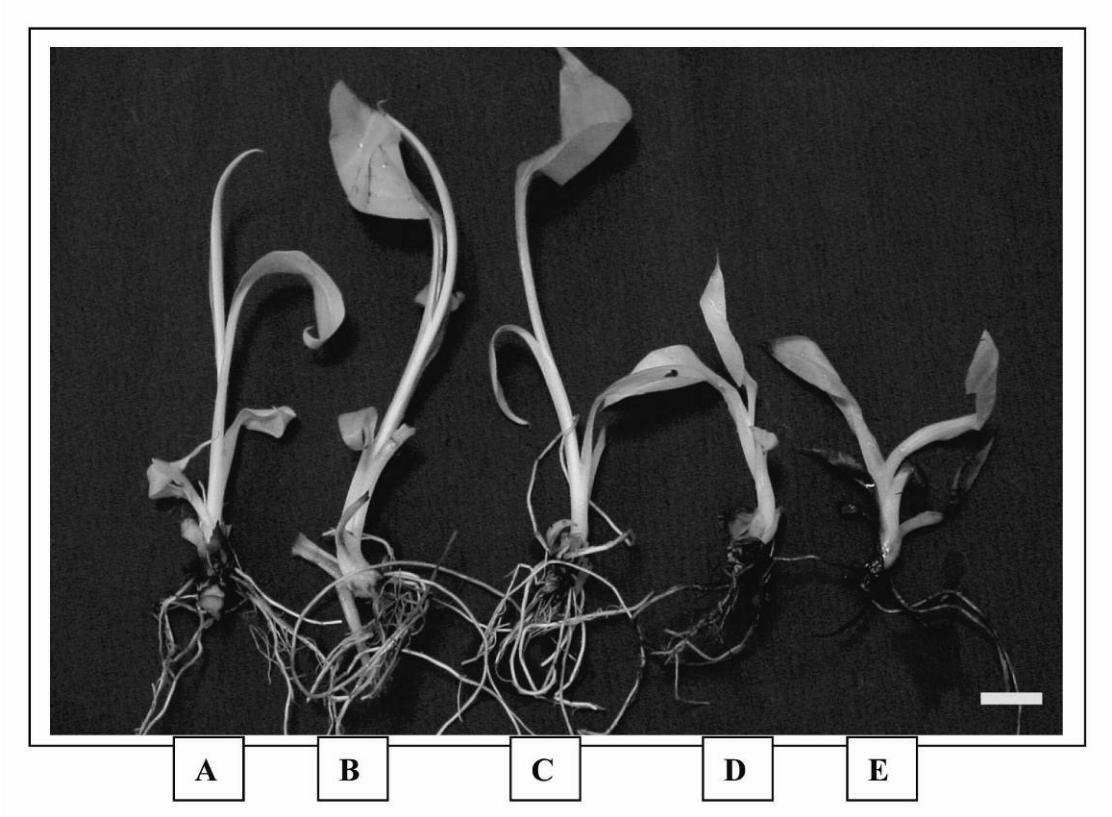
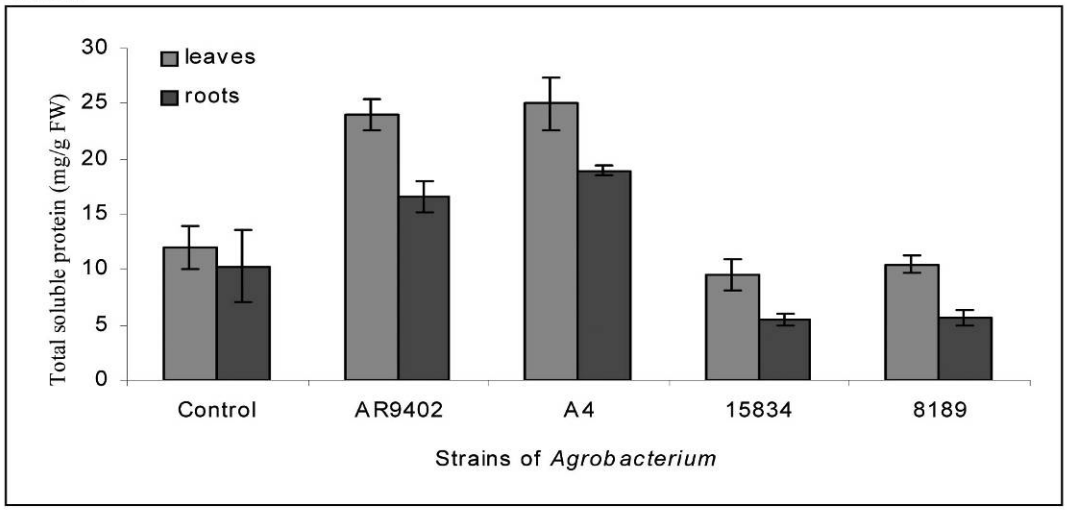
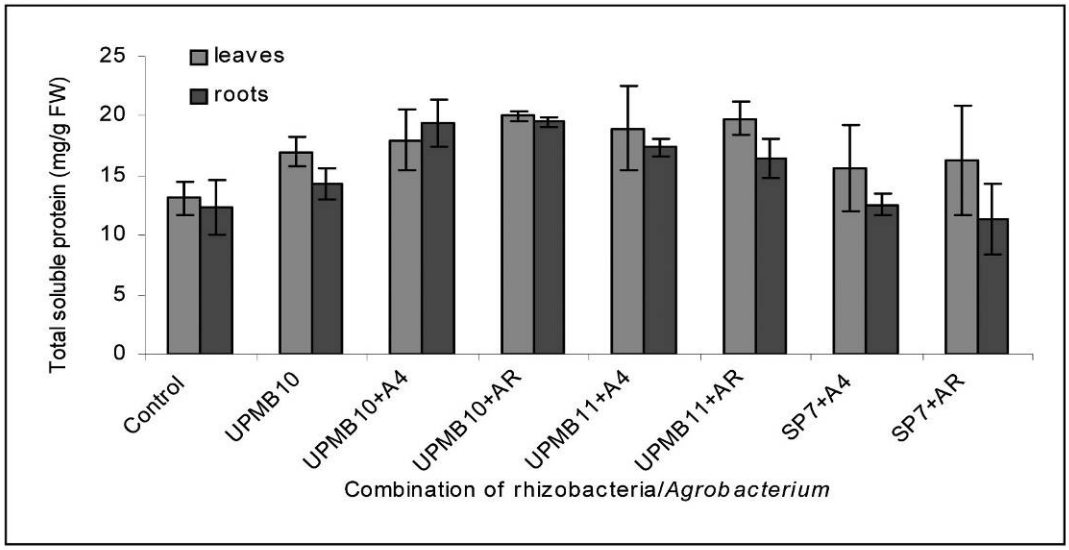


Figure 4. Growth of in vitro banana plantlets after one month culture in MS liquid medium inoculated with agrobacterial: A (control), B (Agrobacterium strains AR9240), C (Agrobacterium strains A4), D (Agrobacterium strains 16758) and E (Agrobacterium strains 14356). Bar represents 10mm.

Panel a



Panel b



Panel c

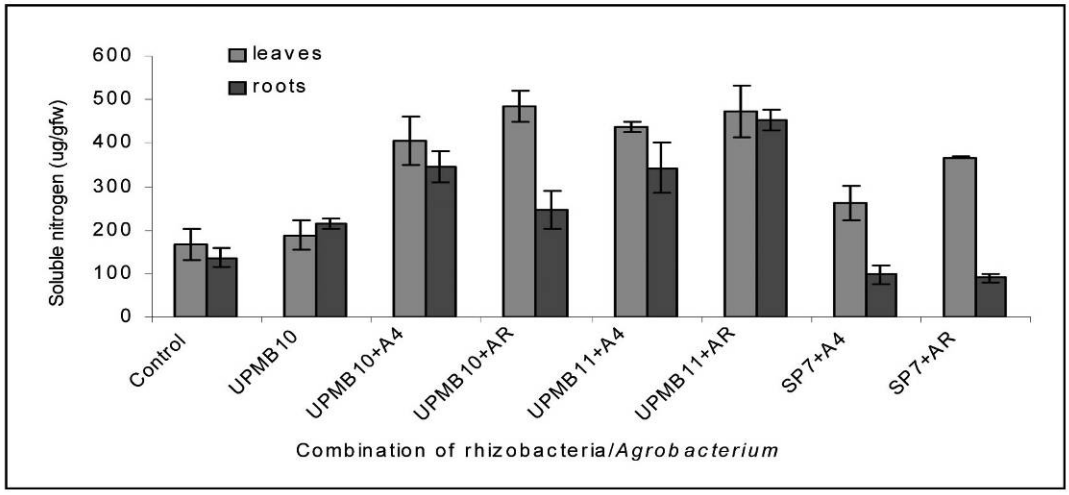
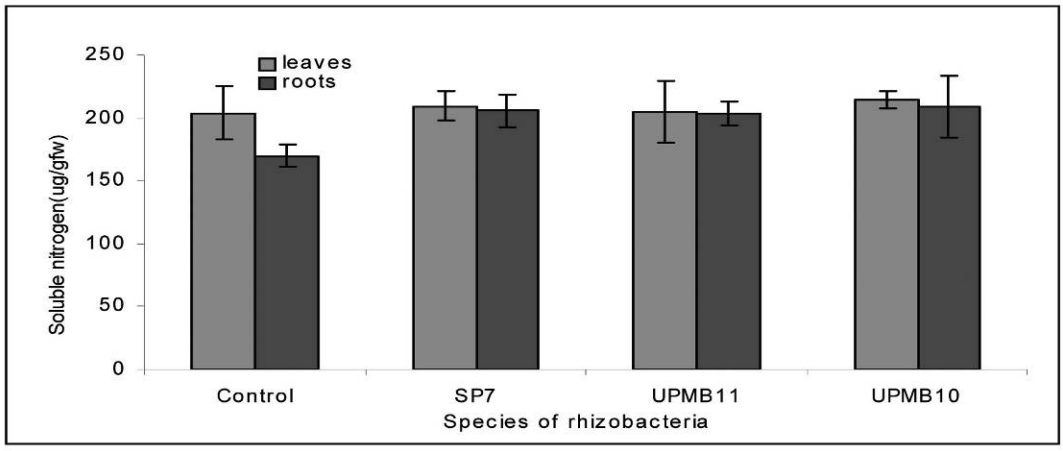


Figure 5. Soluble protein content of in vitro banana plantlets after one month culture in MS liquid medium inoculated with respective bacterial treatment: a (rhizobacteria), b (Agrobacterium) and c (co-inoculation with rhizobacteria and Agrobacterium). Bars show means SD of ten replications (P=0.05, n=10).

Panel a



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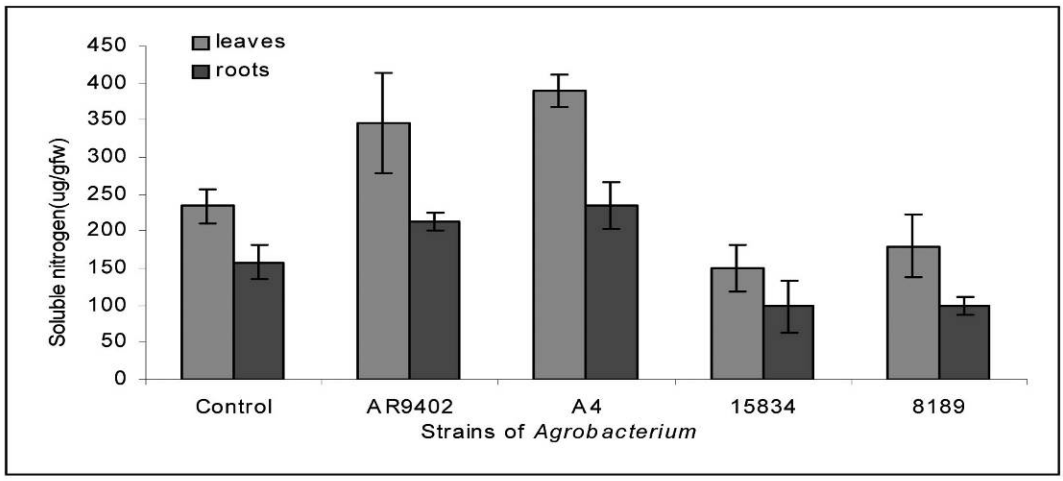


Panel c

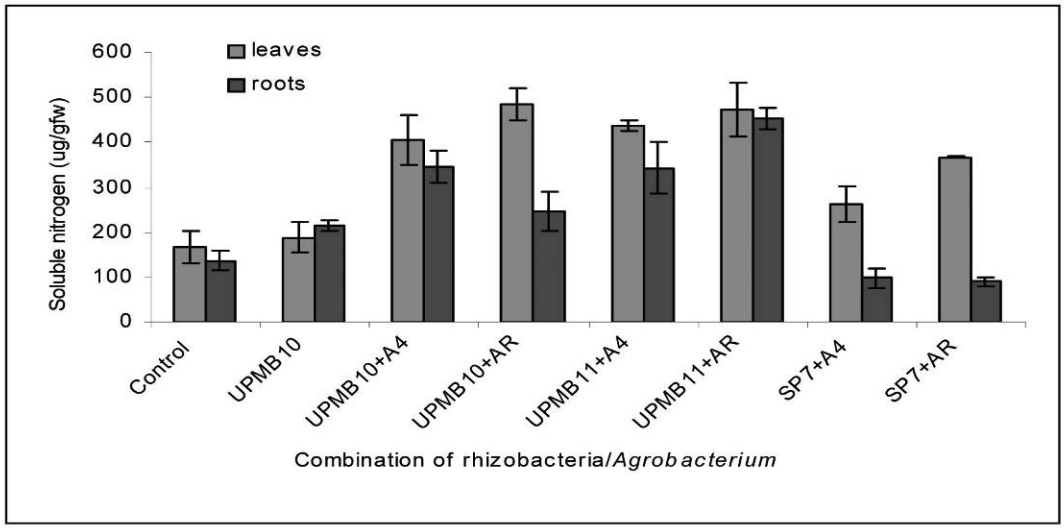
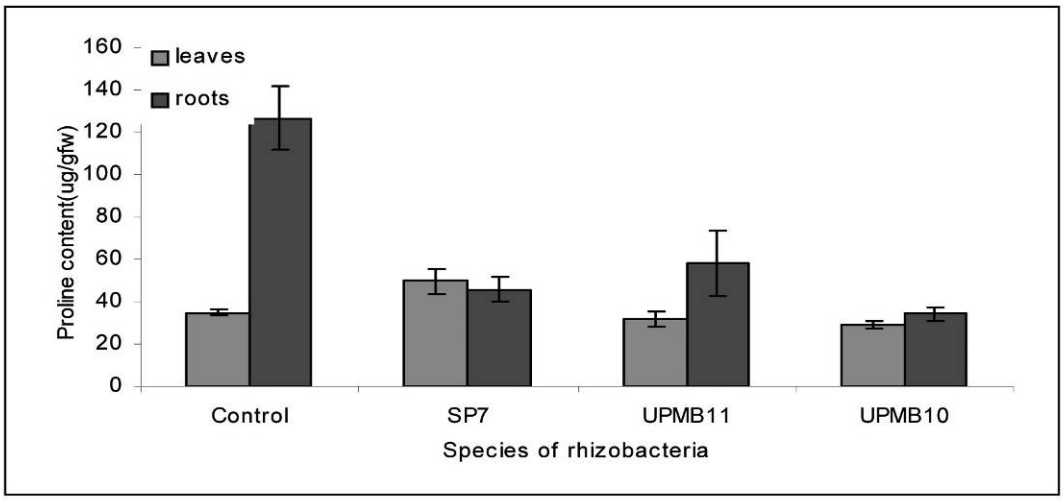
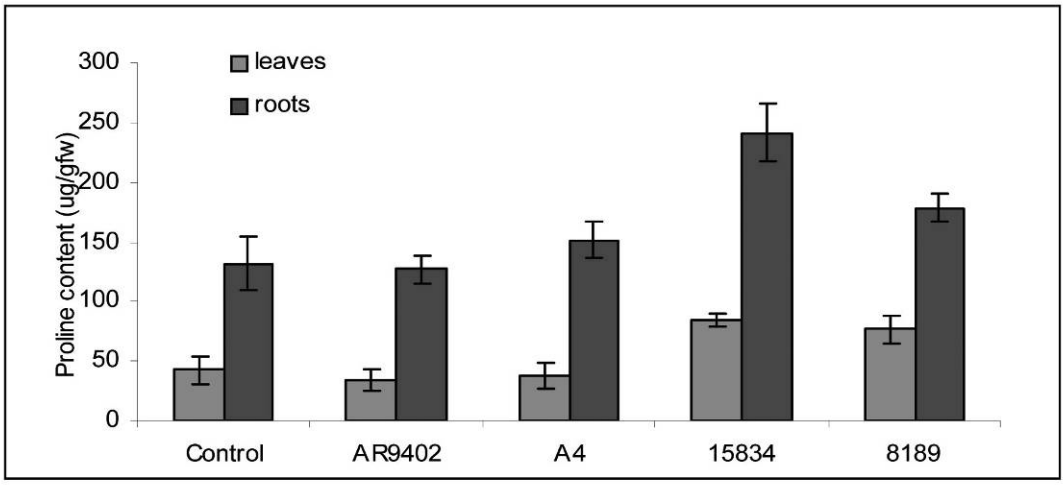


Figure 6. Soluble nitrogen content in part of banana plantlets after one month inoculated with respective bacterial treatments: a (rhizobacteria), b (Agrobacterium) and c (co-inoculation of Agrobacterium) in MS liquid medium. Bars show means SD of ten replications (P=0.05, n=10).

Panel a



Panel b



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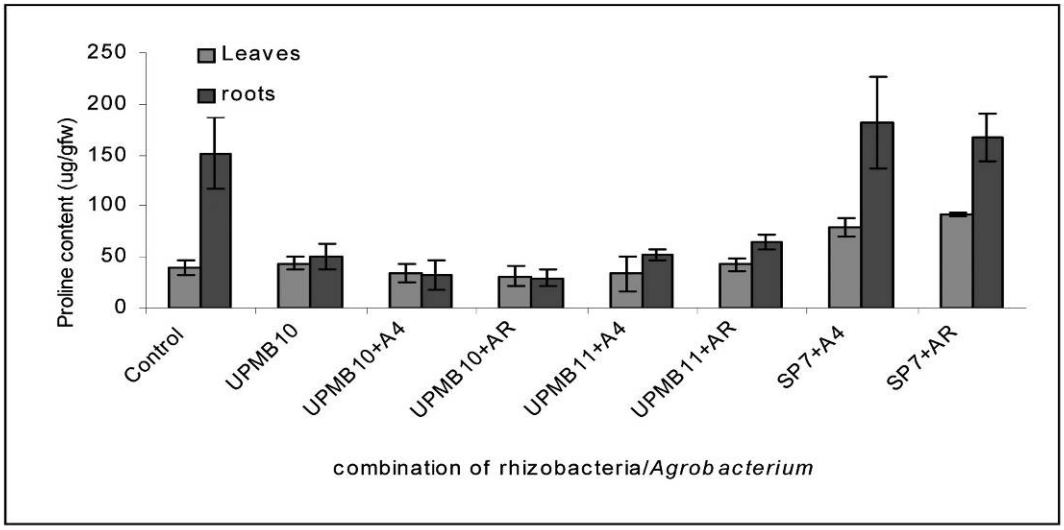
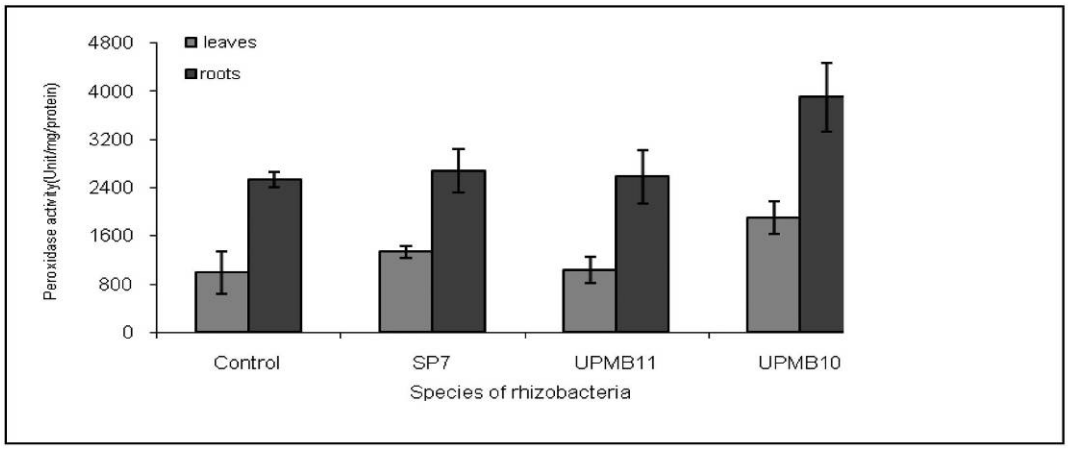


Figure 7. Change of proline content of in vitro banana plantlets for one month culture in MS liquid medium in the presence of respective bacterial treatment: a (rhizobacteria), b (Agrobacterium) and c (co-inoculation of rhizobacteria and Agrobacterium). Bars show means SD of ten replications (P=0.05, n=10).

Panel a



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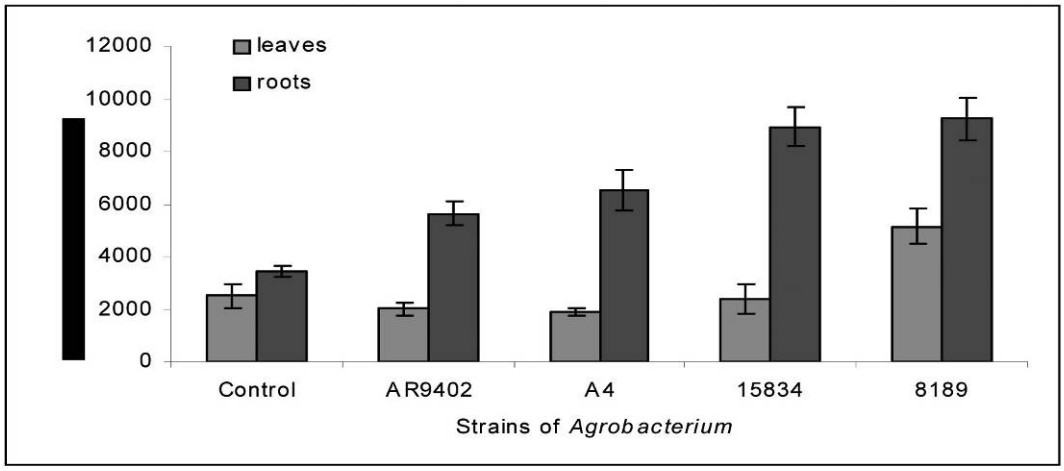

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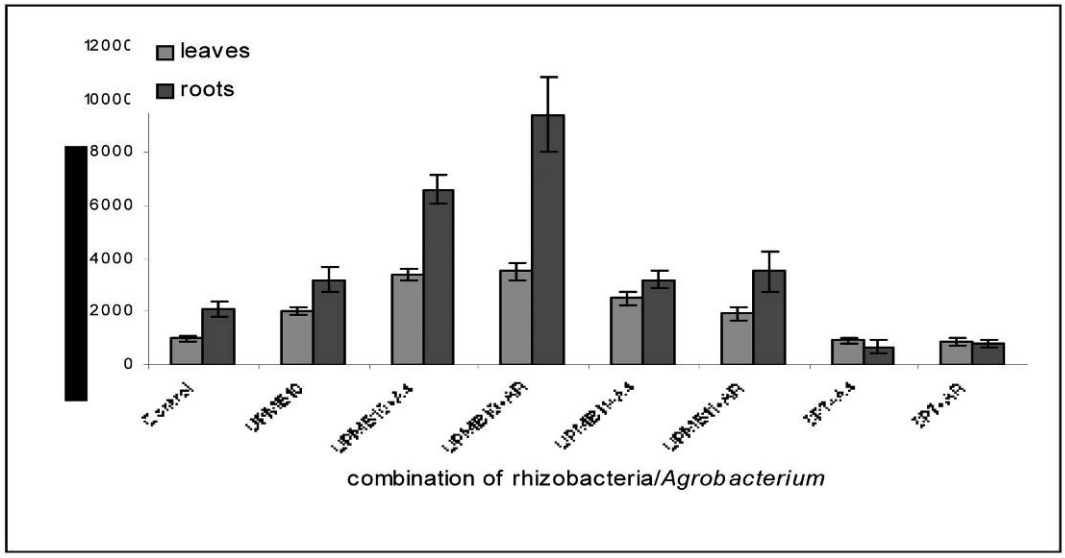
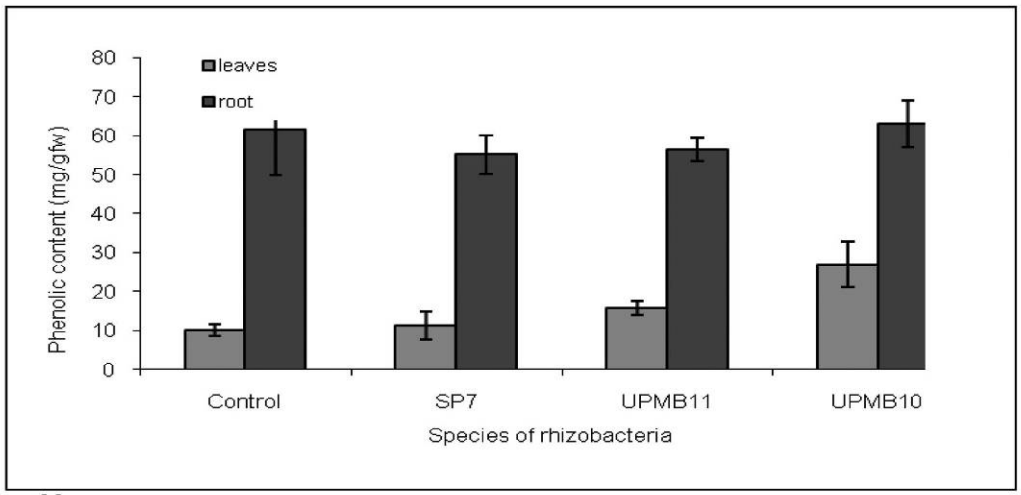


Figure 8. Change of peroxidase activity on in vitro banana plantlets after one month culture in MS liquid medium inoculated with respective bacterial treatment: a (rhizobacteria), b (Agrobacterium) and c (co-inoculation of rhizobacteria and Agrobacterium). Bars show means SD of ten replications (P=0.05, n=10).

Panel a



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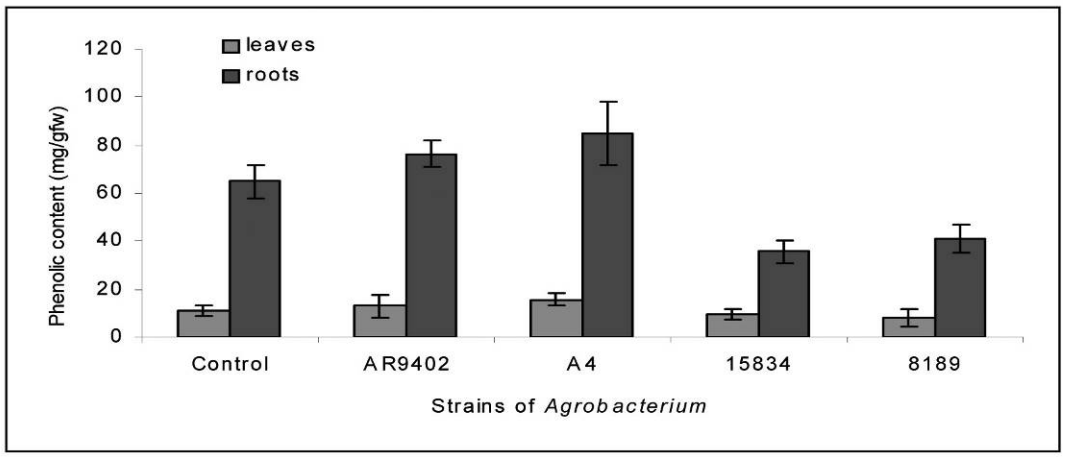


Panel c

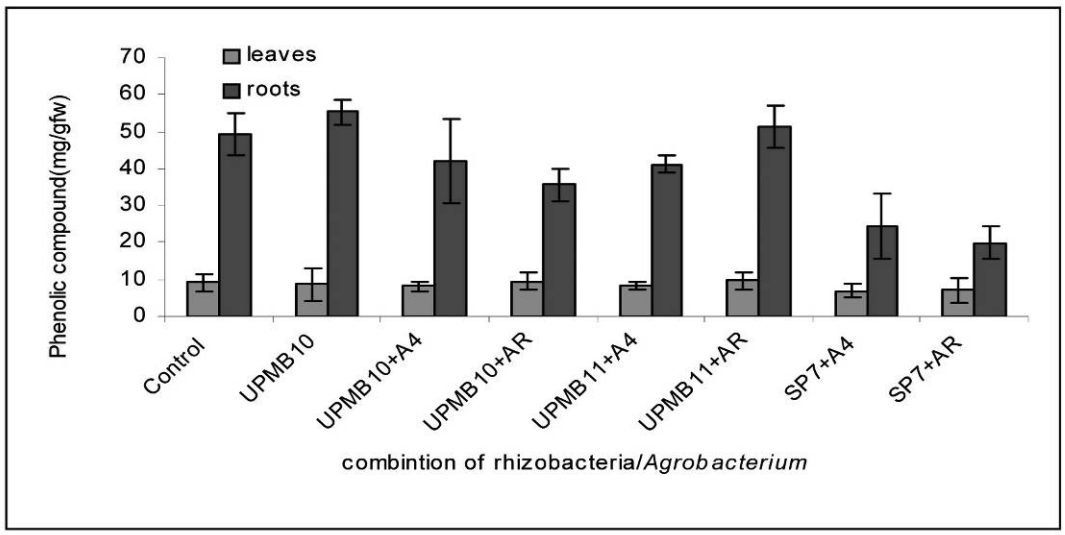
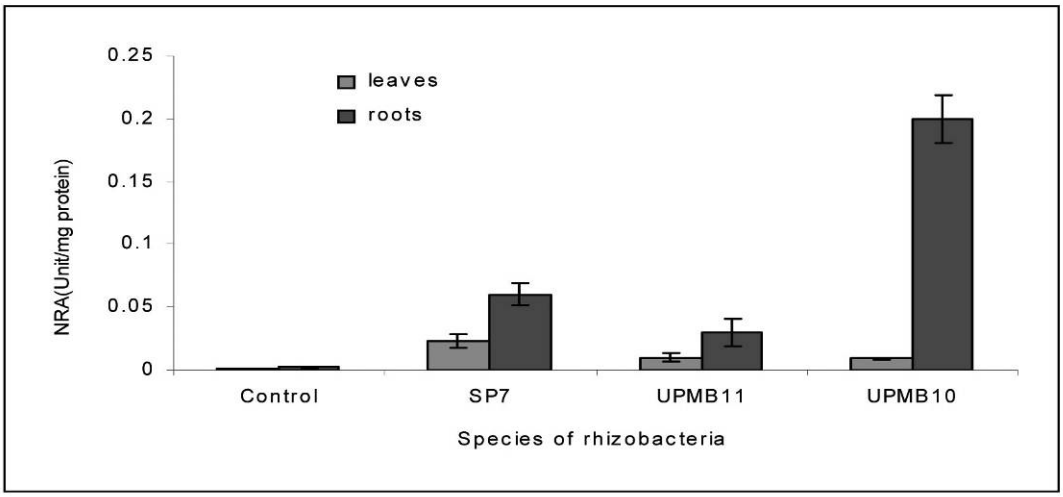
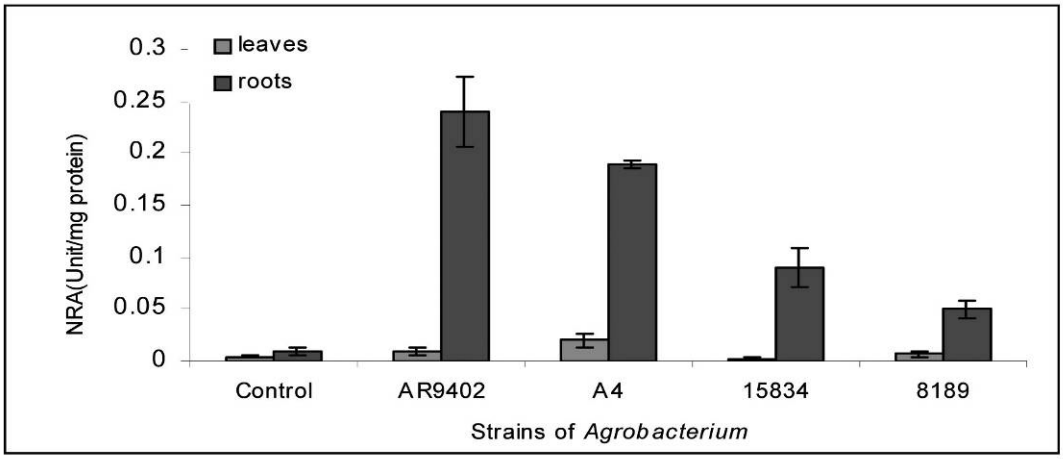


Figure 9. Effect of bacteria: a (rhizobacteria, b (Agrobacterium) and c (co-inoculation of rhizobacteria and Agrobacterium) inoculation on changes of total phenolic compound of in vitro banana plantlets after one month culture in MS liquid medium. Bars show means SD of ten replications (P=0.05, n=10).

Panel a



Panel b



Panel c

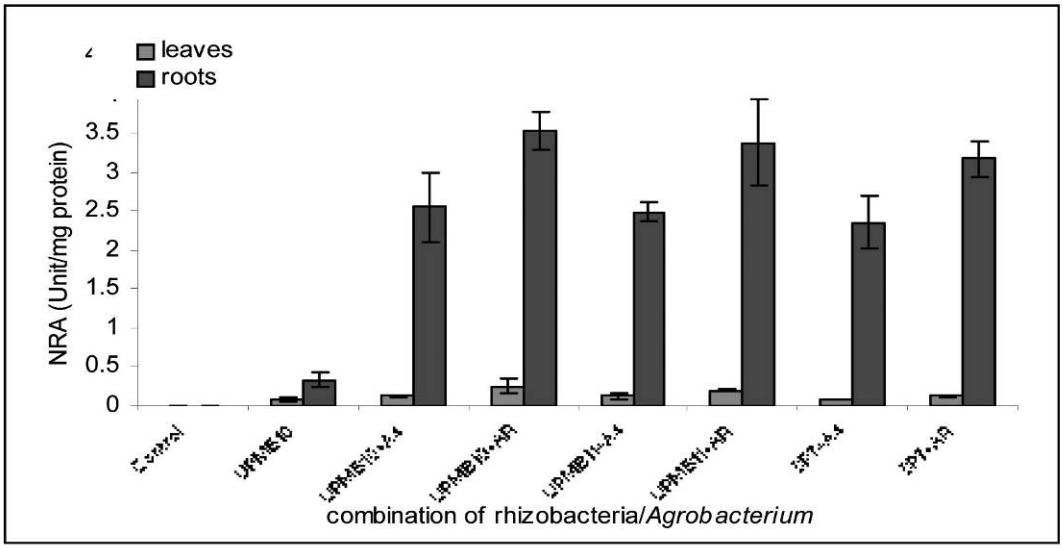
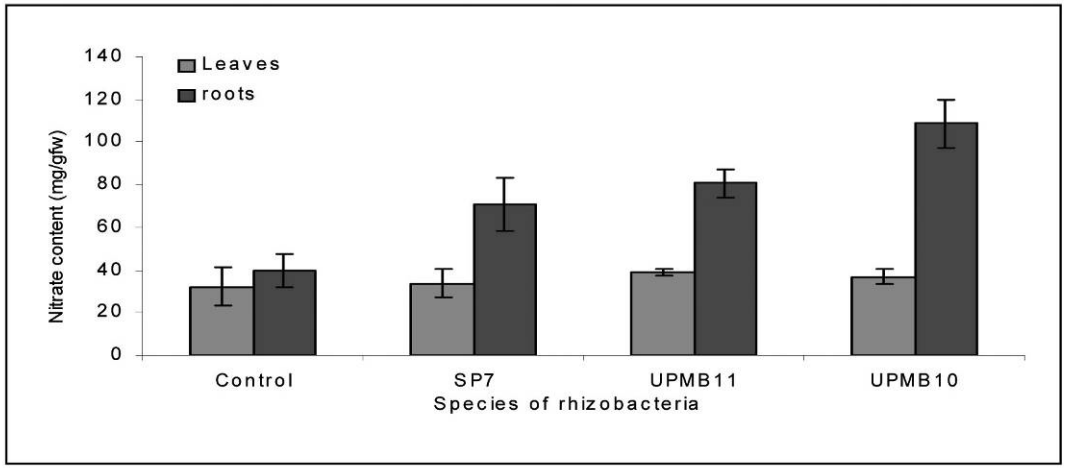
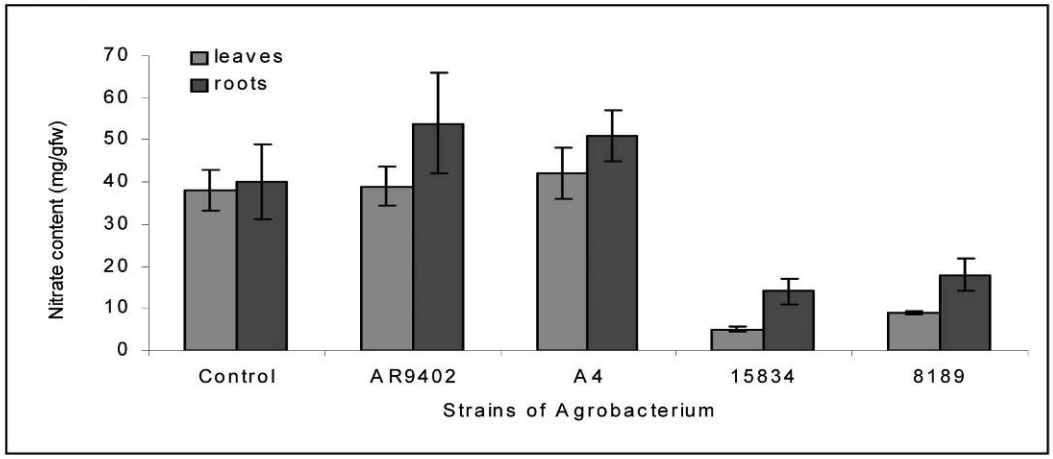


Figure 10. Change of nitrate reductase activity of in vitro banana plantlets after one month culture in MS liquid medium inoculated with respective bacterial treatment: a (rhizobacteria), b (Agrobacteria) and c (co-inoculation of rhizobacteria and Agrobacterium). Bars show means SD of ten replications (P=0.05, n=10).

Panel a



Panel b



Panel c

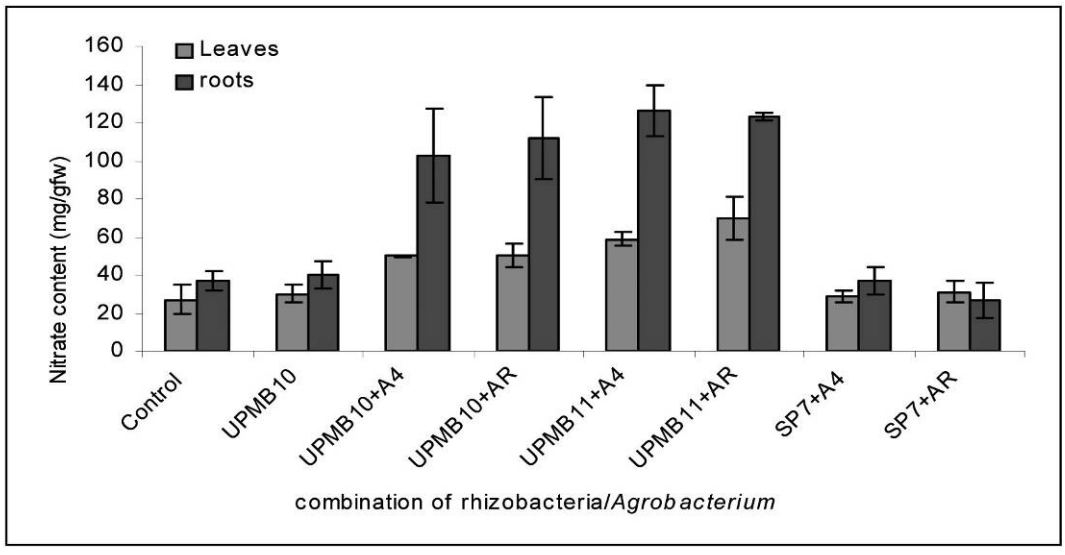
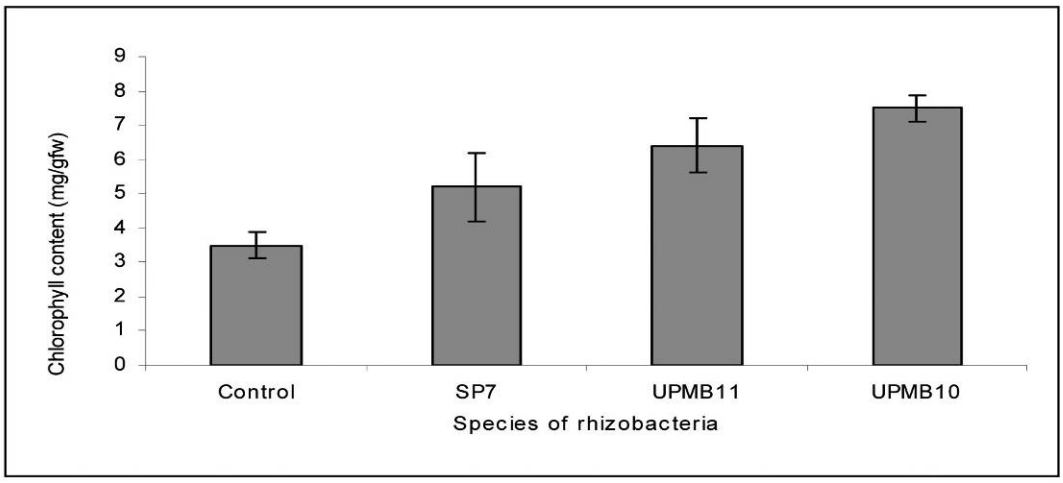
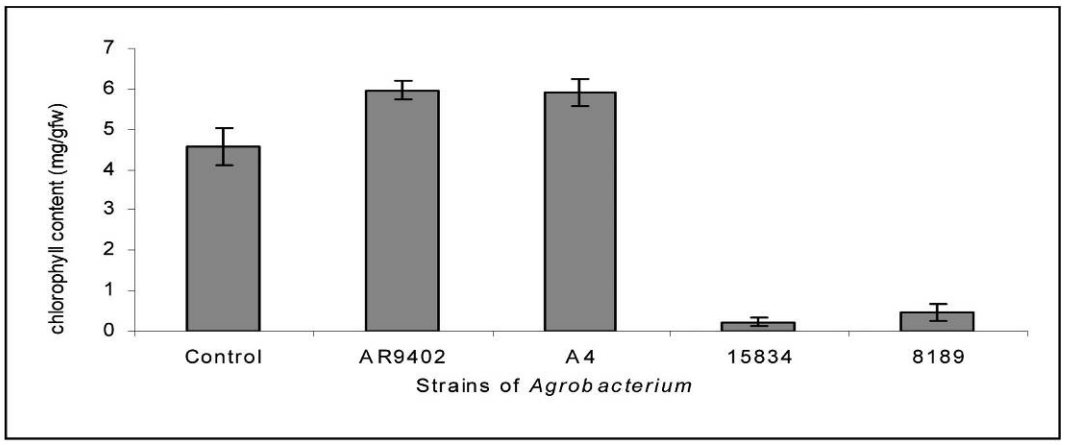


Figure 11. Effect of respective bacterial: a (rhizobacteria), b (Agrobacterium) and c (co-inoculation of rhizobacteria and Agrobacterium) inoculation on nitrate content of in vitro banana plantlets for one month culture in MS liquid medium. Bars show means SD of ten replications (P=0.05, n=10).

Panel A



Panel B



Panel C

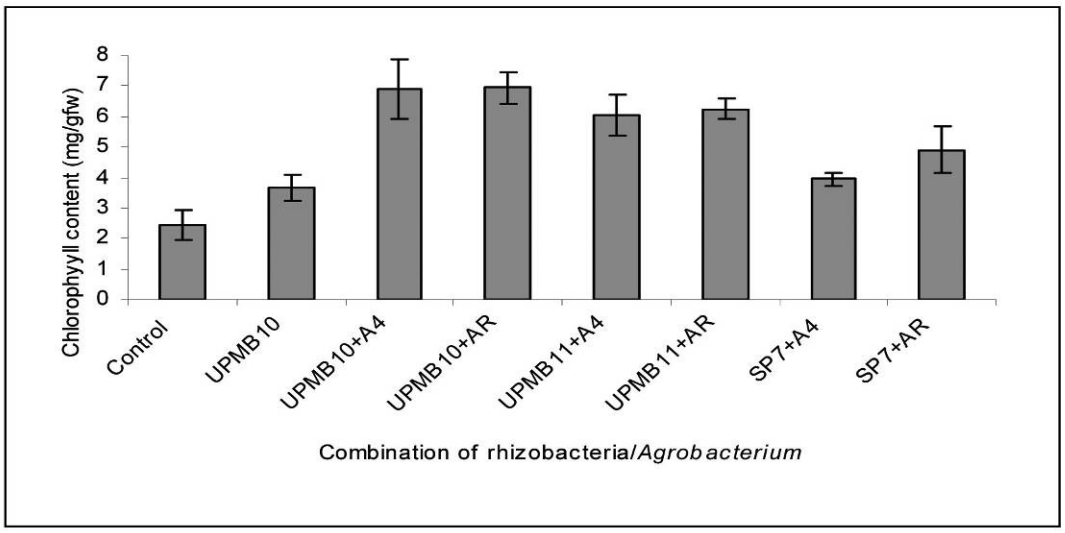


Figure 12. Chlorophyll content of in in vitro banana plantlets after one month culture in MS liquid medium inoculated with respective bacterial treatment: a (rhizobacteria), b (Agrobacterium) and c (co-inoculation of rhizobacteria and Agrobacterium). Bars show means SD of ten replications (P=0.05, n=10).

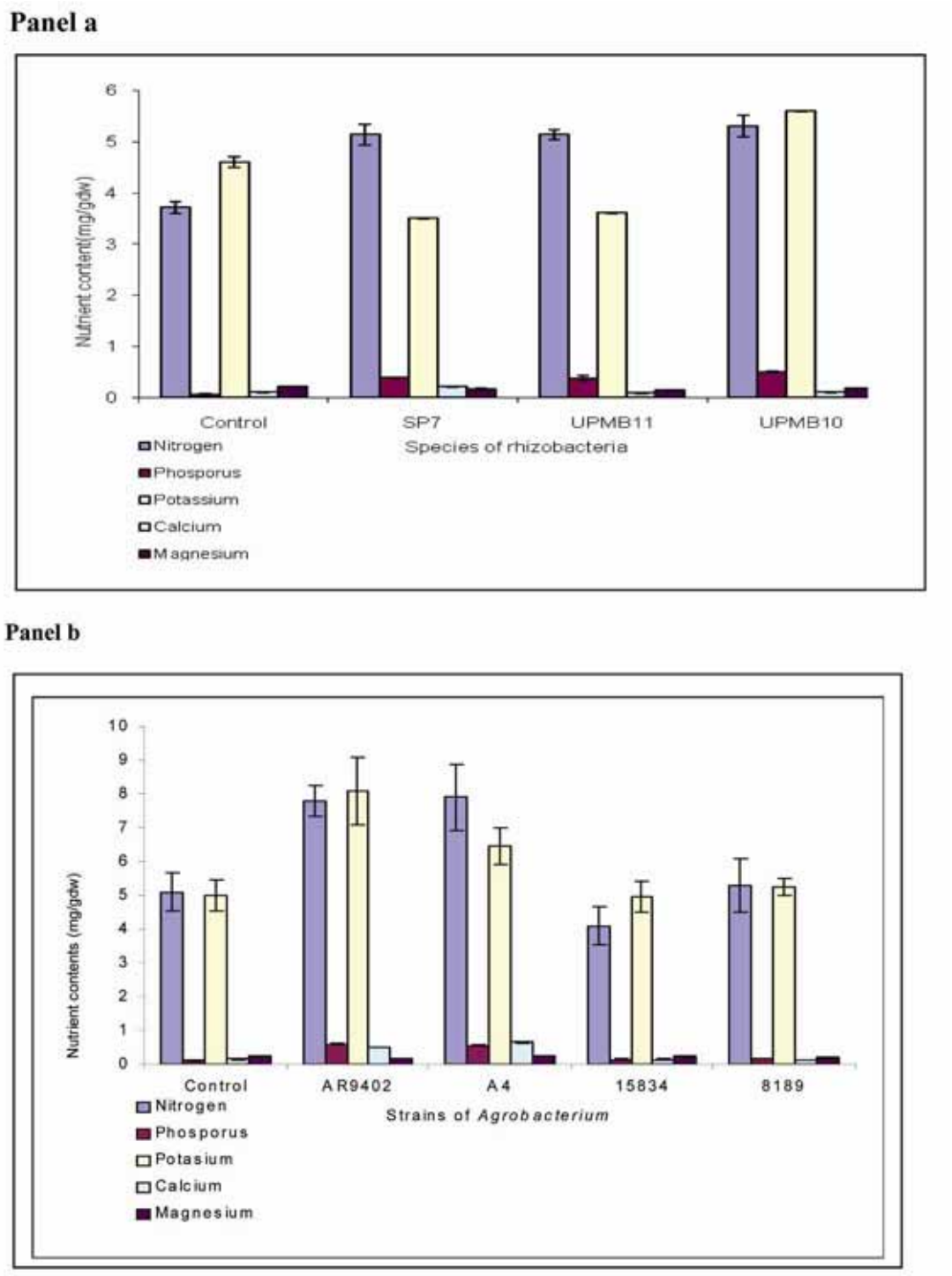


Figure 13. Effect of: a (rhizobacteria), and b (Agrobacterium) on nutrient content of in vitro banana plantlets after one month culture in MS liquid medium. Bars show means SD of ten replications (P=0.05, n=10).

Journal of Agricultural Science
http://www.ccsenet.org/jas
Vol. 2, No. 1
March 2010

Avian Influenza and Employment Decisions of Poultry Farmers in the Federal Capital Territory of Nigeria

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Abstract

The outbreak of Avian Influenza in Nigeria has led to job losses, health problems, reduction in expected income of poultry farmers and a decrease in the demand for poultry products. This study was designed to determine the monetary value of stock lost, identify the determinants of the future employment decisions and the constraints faced by poultry farmers in the Federal Capital Territory of Nigeria. Data for the study was collected from 40 poultry farmers who have suffered losses due to the outbreak of the disease. The total monetary value of the stock lost in the study area as at 2007 was ₦142, 741, 000. 45% of the respondents have abandoned poultry production while 32.5% have reduced the size of their poultry business. Furthermore, only 22.5% have restarted their poultry business without reducing the quantity of the initial stock before the outbreak of the disease. The determinants of the decision to abandon were; amount of compensation received, educational level of the poultry farmer and total number of stock lost. The factors influencing the decision to reduce the scale of operation were; level of education of the farmer, years of experience in poultry production and the amount of compensation received from government. The post Avian Influenza outbreak constraints faced by the farmers were; inadequate compensation, low patronage by customers and low level of accessibility to agricultural credit institutions.

Keywords: Avian influenza, Employment decisions, Poultry farmers, Nigeria

1. Introduction

The importance of poultry production in the national economics of developing countries and its role in improving the nutritional status and income of many small scale farmers and landless communities has been recognized by various scholars and rural development agencies in the last two decades (Adegbola 1990; FAO, 1990). However, the growth in the poultry sector is being threatened by problems facing livestock production which includes high cost of production and outbreak of diseases which recently includes the Avian Influenza or Bird flu. Avian influenza is an acute highly fatal

disease of chickens, turkey, ducts, wild birds, geese and water fowls (Hagan and Brunner, 1988). It is caused by a virus usually found in wild migratory water birds and primarily affects all ages of domestic poultry. Prior to January 2006, there was no report of avian influenza in Nigeria and there was no evidence to suggest the presence of the disease in the country. The first suspected case was reported at Sambawa farms, a semi commercial farm situated in Kaduna State of Nigeria on January 16th 2006 and was finally confirmed on February 7th 2006 by the National Veterinary Research Institute, Vom (Pan-African Control of Epizootics, 2006).

The spread of the disease from three States in March 2006 to 13 States including the Federal Capital Territory as at May 2006 is instructive in the quality and adequacy of response and the capability of the country to deal with the problem. It also suggests that recovery and restricting efforts should not be delayed until the disease is unambiguously stamped out (UNDP, 2006). The incidence of the disease in a country like Nigeria (a country whose most village households maintain free range flocks of poultry as a source of income and food) was a "shock" to poultry farmers, consumers and the economy because the impact ranges from changes in market demand, market supply and finance to external effects (UNDP, 2006). Thus both the productive and consumptive patterns of the rural and urban households are at risk in the event of a virulent disease outbreak such as avian influenza (Obayelu, 2007).

The first reported case of Avian influenza in the Federal Capital Territory Abuja was at Bwari Area Council on the 2nd of February 2006 followed by Kuje area council on the 14th of February 2006 and the Municipal area council on the 15th of February 2006. Various attempts were made to stop the spread of the disease. Olarenwaju (2006) reported that poultry farmers in Nigeria have been affected negatively by the outbreak of avian influenza. Many people refused to eat chicken and eggs to avoid being exposed to the risk of human infection. Moreover, the closure of affected farms has resulted to unemployment. Olarenwaju (2006) quoted the World Bank as saying that a severe avian influenza pandemic may cost the world a whopping $1.25trillion and may result in the death of about 70 million people. You and Diao (2006) analyzed the potential economic impacts of avian influenza in West Africa, taking Nigeria as an example. They concluded that, depending on the size of the affected areas, the direct impact of the spread the disease along the two major migratory bird flyways would be the loss of about 4 % of national chicken production. The study estimates that Nigerian chicken production would fall by 21% and chicken farmers would lose US$250 million of revenue if the worst-case scenario occurred. According to Obayelu (2007) about 75% of poultry farmers in the Federal Capital Territory were found to have stopped ordering for new birds to their farms and were preparing to leave the poultry business for other jobs the moment they disposed off the birds on their farms. About 22, 810 birds have been depopulated as at the end of 2007 from 609 farms and a compensation of over ₦11, 472.800 has been paid to the farmers in the Federal Capital Territory alone {at ₦250 per chicken} (Abuja Echo, 2007). It was estimated that farmers lost millions of naira and many workers lost their jobs thus adversely affecting their dependants in form of drop out of schools, starvation, social inactiveness and quarrels in various families. (Abuja Echo, 2007). Saidu et al (2008) reported that a total of 480,378 birds were lost in 34 outbreaks in four states under study between the period of January and March 2006. Chickens accounted for more than 99% of all the birds affected followed by guinea fowls and turkeys. More than 60% of the birds affected were adults. Raufu et al (2009) also reported a significant difference in the socio-economic and level of income of affected and unaffected poultry farms in Ogun State, Nigeria. Fasina et al (2009) in their study on Avian Influenza Risk Perception among Poultry Workers in Nigeria observed that the respondents were more concerned about the effect of Avian Influenza on financial preservation of business interests than on public health risk.

Further more while various efforts since the outbreak of the disease has contributed on issues of prevention, control and eradication and more importantly prevention of human cases, little attention has been paid to the future employment and production decisions of affected poultry farmers. The aim of this study was therefore to assess the employment decisions of poultry farmers after the outbreak of avian influenza. The specific objectives were to estimate the monetary value of poultry birds lost to the disease, determine the post avian influenza outbreak employment options of poultry farmers, identify the factors that determine the employment options of the poultry farmers and to identify the post avian influenza outbreak constraints in the study area.

2. Materials and methods

The study was conducted in the Federal Capital Territory (FCT) Abuja, Nigeria. The area covers an area of about 8,000 Square Kilometers and has population of 1,405,201 persons (NPC, 2006). The FCT's natural endowment such as its rolling hills, isolated land and endearing feature makes it a delight. The savanna grassland of the north and the middle belt, the richness of the tropical rain forest of the south and an equatorial climate all combined; make the FCT a soil rich agricultural heaven (FCT ADP, 2007). Four area councils where there was a reported outbreak of Avian Influenza namely Gwagwalada Area Council, Municipal area Council, Bwari Area Council and Kuje Area Council were purposively selected for the study. 10 poultry farmers who suffered losses were randomly selected from each of the affected Area Councils. Therefore a total of 40 poultry farmers were used for the study. Primary data were used for the study; this was collected with the aid of a structured questionnaire which was administered to the poultry farmers. Data were collected over a period of 2 weeks by trained enumerators. Data were collected on farmers' socio-economic characteristics,

monetary value of stock lost, employment options and post avian influenza outbreak constraints. Data analysis was done with the aid of descriptive statistics and Multinomial logit model. The model was fitted and estimated using multinomial logistic regression. A multinomial logistic regression pinpoints determinants affecting the probability for the choice of the future employment decisions of the poultry farmers. The dependent variable reflects the three likely employment decisions: continuation with poultry production, abandoning of poultry production, and reduction of the scale of production. The dependent can thus take three levels (0, 1 and 2). '0' represents the reference group or the group that continued production; '1' represents the group that abandoned production and '2' represents the group of those that have reduced their scale of poultry production. According to Maddala (1983), the model makes the choice of probabilities on individual's characteristics of the respondents (poultry farmers). Following Babcock *et al*., (1995), the basic model was specified as follows;

$$P_{ij} = \frac{e^{\beta_j X_i}}{\sum_{K=0}^{j} e^{\beta_j X_i}}$$

where i=1, 2-------n variables; k=0, 1,-------j groups; and β_j = a vector of parameters that relates the X_i's to the probability of being in group j where there are j+i future employment decisions. In this study, X_1 to X_5 are independent variables that may influence the future employment decision of a poultry farmer.

Where;

X_1 = Years of formal education of the poultry farmer (years)

X_2 = Age of farmer (Years)

X_3 = Years of experience in poultry production (years)

X_4 = Total amount of compensation received (₦)

X_5 = Quantity of stock lost (actual number)

3. Results and discussion

3.1 Monetary value of stock lost and future employment option of poultry farmers

The monetary value of stock lost due to the outbreak of Avian Influenza in the study area is presented in Table 1. The outbreak was highest in Kuje followed by Bwari Area council. The future employment option of the poultry farmers is presented in Table 2. The table revealed that majority of the poultry farmers have either abandoned or reduced the scale of their poultry business. Majority of the poultry farmers decided to diversify from poultry business due to the inadequate amount of compensation received from government as well as reduced patronage by the customers after the outbreak of Avian Influenza.

3.2 Factors that determine the future employment decisions of poultry farmers

The Factors that determine future employment decisions of the poultry farmers are presented in Table 3. The results revealed that the likelihood ratio test for the model Lambda (λ) was 12.76 and was significant at 5%. This means that the future employment options of the poultry farmers are heterogeneous. This finding confirms the appropriateness of the polychotomous model in the study. The table also revealed that three variables are likely to increase the probability of the farmer to decide on reducing the scale of production namely; level of education of the farmer, years of experience in poultry production and amount of compensation received. This implies that the three variables are associated with a higher probability of being in the choice group under consideration relative to the reference group. Furthermore, farmers' level of education and amount of compensation received are likely to be associated with the probability of a poultry farmer deciding to abandon poultry production relative to the reference group. As expected well educated farmers are likely to have other types of trainings which can enable them invest outside the poultry sector. The amount of compensation received will also go along way in helping to re-start the business. However if the compensation is inadequate, poultry farmers will not be encouraged to take the risk again. The most likely decision will be to abandon the poultry business for a less risky one.

3.3 Post Avian Influenza outbreak constraints

The post avian influenza outbreak constraints are presented in Table 4. Reduced patronage by customers, inadequate compensation and lack of access to formal agricultural credit were the major constraints noted in the area. Among the problems reported, reduced patronage by the customers was ranked first followed by inadequate compensation.

4. Conclusion and recommendations

The result of this study showed that many poultry farmers have abandoned the poultry business while some have reduced the scale of their poultry businesses. Abandoning of poultry business and reduction in the scale of poultry production will result in low production of poultry products with an attendant effect on the availability and prices of poultry products. Giving the importance of poultry production as a source of income and employment for many Nigerians, the followings are recommended.

- There is an urgent need for Government to mandate Poultry Farmers to take up Insurance policies with the Nigerian Agricultural Insurance Cooperation. This will help to guarantee adequate compensation at all times.
- Formal agricultural credit institutions who attempt to discriminate against poultry farmers should be sanctioned by the Central Bank of Nigeria.
- Efforts should be intensified in the area of public enlightment and dissemination of up-to-date information on avian influenza infection and control in humans in order to restore consumer confidence.

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Table 1. Monetary value of Poultry Birds lost to Avian influenza

Affected Area councils	No of birds	Unit price (₦)	Total (₦)
Gwagwalada	14 592	1000	14 592 000
Municipal	20 134	1000	20 134 000
Bwari	38 248	1000	38 248 000
Kuje	69 767	1000	69 767 000
Grand Total	**142 741**		**142 741 000**

₦154 = 1USD

Table 2. Future employment options of poultry farmers

Future employment option	Frequency	Percentage
Continuing producing poultry	9	22.5
Abandoning poultry business	18	45.0
Reduction of scale of operation	13	32.5
Total	40	100.0

Table 3. Multinomial logistic regression model of future employment decisions of Poultry farmers

Variables	reduction of scale of production	abandoning of poultry business
	Parameters	Parameters
Education (X_1)	0.280 (0.532)*	0.668(0.432)**
Age (X_2)	0.461 (0.631)	0.178 (0.575)
Years of Experience in Poultry Production (X_3)	0.393 (0.748)***	0.294 (0.656)
Amount of Compensation received (X_4)	2.001 (0.00)*	1.133(0.000)*
Quantity of stock lost (X_5)	5.599 (0.000)	3.579 (0.000)*
Constant	2.610 (1.986)	3.317 (2.13)
Log likelihood	-72.812	
Likelihood Ratio (λ)	12.74*	
n	40	

Note: Figures in parenthesis are the standard error of the estimated coefficients

*Significant at 10% level of Significance

** Significant at 5% level of Significance

*** Significant at 1% level of Significance

n = sample size

Table 4. Post Avian influenza outbreak constraints

Constraints	**Frequency**	**Percentage**	**Rank**
Reduced patronage by customers	37	33.04	1st
Inadequate compensation	33	29.46	2nd
No compensation	11	9.82	4th
Difficulties in obtaining formal Agric. Credit	31	27.68	3rd
Total	112*	100.00	

* Multiple response was allowed, hence total frequency exceeded the total sample size

Journal of Agricultural Science
http://www.ccsenet.org/jas
Vol. 2, No. 1
March 2010

Statistical Analysis of Main and Interaction Effects to Optimize Xylanase Production under Submerged Cultivation Conditions

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Abstract

In recent years, xylanase has become an essential option for environmental friendly industrial biotechnological applications and there is a rising demand for large scale production. In this study, a *Bacillus species* 2129 was tested for the xylanase production under submerged cultivation conditions. Maximum xylanase activities were achieved using oat as the substrate and by optimizing process conditions such as substrate concentration, pH and nitrogen source using statistically significant design of experiments, employing the response surface methodology (RSM) concept. Under optimized conditions there was an 8% increase in the enzyme activity and results from statistical approximation in the form of analysis of variance (ANOVA) shows that the squared effects of the variables were significant than both the main and interaction effects.

Keywords: Oat, Xylanase production, Response surface methodology, Optimization

1. Introduction

Xylanase is a hemi – cellulolytic polysaccharide consisting of 1,4 linked β–D–xylo pyranose residues, most commonly used for beer and juice clarification, pre–bleaching of kraft pulp, improving digestibility of animal feed, bread making and degumming of vegetable fibers such as jute ramie and hemp (Senior *et al.*, 1992; Bocchini *et al.*, 2005). The main constituents of microbial xylanolytic enzyme system are xylanase (endo–1,4–β–xylanase) and β–xylosidase (β–D–xyloside xylohydrolase) (Heck *et al.*, 2005). Over the last few years, interest in xylanase has increased rapidly in paper and pulp industries due to their bleaching potential. Xylanases have a worldwide market of around 200 million US $ and the widespread use of xylanase in commercialized industrial applications requires extensive studies to optimize their production capability (Sonia *et al.*, 2005). There has been extensive lab and pilot scale studies that have dealt with their production, purification, recovery and characterization (Jain, 1995; Sa–Pereira *et al.*, 2002; Seyis and Aksoz, 2005; Shah and Madamwar, 2005). On the other hand, very few studies have reported their product optimization (Narang *et al.*, 2001; Senthilkumar *et al.*, 2005; Heck *et al.*, 2005). Commercial mass production of xylanase can be quintessentially done by either submerged or solid state fermentation (Pandey, 1992; Bocchini *et al.*, 2005), their effectiveness has been often consociated with process conditions and physico–chemical factors of prior significance. However the driving force has been to ideally produce quick and high quality xylanase from simple and inexpensive substrates. Most of the researches have been targeted on using residues/wastes from agro and food industry, thereby restricting the socio–economics related to environmental pollution. These residues contain nearly 20–30% hemicellulosic material that can be efficaciously used for the production of xylanase by microorganisms (Milagres *et al.*,

2004). The most commonly used substrates so far are; rice bran, sugarcane baggase, wheat straw, wheat bran, corn crop, rumen, sorghum straw and cassava peel (Alam *et al.*, 1994; Wang *et al.*, 2003; Sonia *et al.*, 2005; Oliveira *et al.*, 2006).

In this study, commercially available Oat was used as the substrate for the production of xylanase under submerged conditions. Oat grains (*Avena sativa*) are high in carbohydrates and contain about 13% protein and 7.5% fat. Studies that have reportedly used oat as the substrate for the production of xylanase are sparse (Oliveria *et al.*, 2006). Diversified generic species of microorganisms have proven to be carriers of rich source of xylanase enzyme, especially *Bacillus species* which can secrete high levels of extra cellular xylanase. The amount of nitrogen also plays a vital role in enhancing the rate of enzyme production. NH_4NO_3, $NaNO_3$ and $(NH_4)_2SO_4$ have been used essentially as the nitrogen source (Abdel–Sater and El–Said, 2001). Seyis and Aksoz (2005) used a mixture of NH_4SO_4 and urea and found synergistic increase in xylanase activity. For successful implementation of this new substrate, process parameters such as pH, temperature, substrate concentration, cultivation and aeration time has to be optimized in appropriate reactor configurations. Though there are different optimization tools, factorial experiments and response surface methodology provides maximum information based on statistical principles by performing a minimum number of experiments (Montgomery, 1991).

This paper reports the optimization of substrate concentration, pH and nitrogen source for enhanced production of xylanase by a *Bacillus species* under submerged fermentation conditions.

2. Materials and methods

2.1 Microbial strain

The microbial culture used in this study was *Bacillus sp*. 2129, obtained from National chemical laboratory (NCL), Pune, India. Stock cultures were maintained on slants of nutrient agar medium at 4 ^oC and were periodically sub cultured to sustain microbial activity.

2.2 Media composition

The minimal medium used in this study had the following composition (per liter): beef extract – 1g, peptone – 1g and Sodium chloride – 0.5 g. Oat, obtained commercially from Quakers Company was used as the substrate (carbon source) at varying concentrations (0.52 – 2.87 %). All other chemicals used in this study were of analytical reagent grade purchased from Sigma Laboratories (India). The values of NH_4Cl and Oat concentration are expressed in %, in (*weight/volume*) basis.

2.3 Experimental study

Experiments were conducted in 250 ml Erlenmeyer flasks fitted with butyl rubber stoppers having a working volume of 100 ml. The individual experimental flasks containing the media were sterilized at 15 *psi*, 121 ^oC for 20 minutes prior to inoculation. *Bacillus species*, maintained on nutrient agar slants were grown for 3 days at 30±1^oC. After sufficient growth, 10ml of distilled water was aseptically added to each agar slants. Through mild scrapping with a sterilized inoculation loop and by periodic shaking, the colonies were made to suspend. For growth, 200 µl of this suspension was aseptically transferred and provided as the inoculum to the 100 ml media. The *Bacillus* strain was grown in experimental flasks kept in a rotary shaker (150 rpm) at 30±1^oC and sample aliquots were withdrawn at equal intervals (12 hrs) for measuring xylanase activity.

2.4 Enzyme activity measurements

Xylanase activity was measured by monitoring the reducing sugar concentration released as xylose by the dinitrosalicylic acid (DNS) method (Miller, 1959). The samples were centrifuged at 7200 rpm for 15 mins and used for analysis. 0.1 ml of this sample was mixed with 0.9 ml of birchwood xylan solution (5 g/l) in acetate buffer (0.1 M) having a pH of 5.0 at 60^oC for 10 mins. The absorbance was read at 550 nm using a UV/Vis spectrophotometer (Shimadzu, Japan). A unit of xylanase activity was described as the amount of enzyme producing 1 µmol of reducing sugar equivalent to xylose per minute under standard test conditions.

2.5 The 2^3 central composite design

To investigate the effect of parameters such as substrate concentration, pH and ammonium chloride concentration on the enzyme activity, experiments were carried out according to the full factorial central composite design (CCD) as described by Montgomery, (1991). The three steps of this experimental design include statically designed experiments, estimating the coefficients in a mathematical model and predicting the response and checking the applicability of the model. A 2^3 CCD for three independent variables, each investigated at five levels with six star points and six replicates at the central point was experimented to fit a second order polynomial model that required 20 experiments. The number of center point runs that the design specifies depends on certain inherent properties required for the design (Montgomery, 1991; Gopal *et al.*, 2002). The start points represent new extreme (low and high) for each factor in the design (Techapun *et al.*, 2002). To maintain rotatability, the value of α depends on the number of experimental runs in the factorial portion of the CCD. If the factorial is a full factorial with "k" factors, then

$$\alpha = \left[2^k\right]^{1/4}$$

The dependent variable (response) selected for this study was the enzyme activity, expressed in U/ml, while the independent variables chosen were oat concentration (X1), media pH (X2) and ammonium chloride concentration (X3). The range and levels of these experimental variables are given in Table 1.

According to the CCD theory, the response variable can be approximated to the process variables by a second order polynomial model of the form:

$$Y=b0+ b1X1+ b2X2+ b3X3+ b11X12+ b22X22+ b33X32+ b12X1X2+ b13X13+ b23X2X3+ \varepsilon$$

Where Y is the measured response, bo the intercept term, b1–3 are the measures of the effects of variables (coefficients) and ε is the experimental error. The test factors were coded according to the following equation.

$$x_i = \frac{X_i - X_i^x}{\Delta X_i}$$

Where x_i is the *coded* value, X_i is the *actual* value of the i^{th} independent variable, X_i^X is the actual value of the i^{th} independent variable at the center point and ΔX_i is the step change value. The MINITAB 14 (PA, USA) software was used for regression and graphical analysis (Response surface and contour plots) of the data obtained. Analysis of variance (ANOVA) was used to estimate the statistical parameters. The predicted values were calculated from the regression model derived from the coefficients of the model and variations were explained by the determination coefficient (R^2 values).

3. Results and Discussion

Experiments were carried out to optimize the effects of various process variables such as initial substrate (oat) concentration, pH and ammonium chloride (NH_4Cl) concentration for xylanase production according to the statistically significant 2^k full factorial central composite design (CCD). Furthermore, the results were analyzed by analysis of variance (ANOVA). This assisted in elucidating the main, squared and interaction effects among the process variables and their influence on the measured enzyme activity. All experiments were carried out in sequential order as specified by the design, in duplicate and the average values of measured enzyme activity were taken as the response variable. Figure 1 depicts the main effects of process variables on the enzyme activity, while Table 2 describes the process conditions and the experimentally measured enzyme activity. It was found that these profiles neither showed a single increasing or decreasing trend, but displayed a combination of both increasing and decreasing trends, suggesting the existence of an optimum condition within the range of experimental study. When the substrate concentration was increased from 0.5% to 1.7% the enzyme activity increased from a value of 0.23 to 1 U/ml, and then decreased to around 0.2 U/ml at a substrate concentration of 2.8772 %. With the increase in pH concentration from low to high levels, the enzyme activity decreased from a value of 2.2 to about 0.45 U/ml and then the enzyme activity progressively increased to a value of 2.2 U/ml. Similarly, on increasing the NH_4Cl concentration, the enzyme activity first showed a declining trend and then increasing trend at a concentration of 0.6681%. The maximum enzyme activity was achieved for the substrate concentration of 1.7 %, pH of 6.807 and NH_4Cl concentration of 0.5 % (run number 11). The xylanase production was between 0.48–0.49 U/ml in the medium with the three test variables at their central level. The carbon source used in this study is one of the major factors affecting the production of enzymes and their levels. The graphical representation of the interactions between process variables and their response called the response surface plots (RS plots) are presented at different levels of substrate concentration, pH and NH_4Cl concentrations in Figures 2, 4 and 6. Each contour plot showed an infinite number of combinations of the two test variables with the other variable maintained at '0' level. The peaks and curvature indicated the maximum enzyme activity in the RS plots. The shapes of the surfaces, circular (or) elliptical indicated whether the interactions among different variables were significant or not. In general, the RS plots can be dome shaped, inverted 'U' shaped, some with a saddle point and some do not show any regular variation with increase / decrease in variables. Each RS plot is further complimented with contour diagrams (Figures 3, 5 and 7) that reveal information on the variation of response on a XY plane. The surface confined to the smallest curve of the contour diagram suggested the location of an optimum operating condition under the experimental condition. The graphical illustrations in Figures 2 and 3 reveal that the enzyme activity was at its maximum under the following condition: high pH and low levels of NH_4Cl, high NH_4Cl and low pH. These plots showed a shallow surface of an optimum condition at the intermediate levels and hills at extreme operating conditions. This interaction behavior causes the optimal level of one variable to change in response to changes in other variables. Mathematically, a saddle point (Figure 4) is a point of a function with two or more variables which is at stationary point but not extremum. At such point, the surface could resemble a saddle point that curves up in one direction and curves down in one or several other directions. The saddle shaped surface does not have a unique optimum; instead it represents maximum value of the response variable in one direction, but a minimum in one or several directions. As shown in Figure 4, the rate of increase in enzyme activity with an increase in value of pH above its saddle point is greater than the rate of increase in

enzyme activity when substrate concentration is increased/decreased from the saddle point. Similarly, Figure 6 shows that higher enzymatic activity is achieved by decreasing NH_4Cl below its saddle point value than by increasing it above its saddle point value.

Further, the contour plot (Figure 3) depicts concentric elliptical ridges within the design boundary, and this runs diagonally from the lower right to the upper left end. These types of contours passes through the steepest ascent of enzyme activity and the optimum operating conditions and in direction of maximum decline of the response with respect to increasing or decreasing values of the process variables. The interactions between substrate concentration and pH (Figure 5) showed a rather complex behavior in comparison to those explained earlier. At low and high levels of pH, increasing the substrate concentration increased the enzyme activity up to a maximum and then decreased their values. However at intermediate levels of pH, irrespective of the values of substrate concentration, there existed a region where neither an increasing nor decreasing trend in the enzyme activity was noticed. These are represented by complex saddle type contour plot as shown in Figure 5. Similar type of interactions was observed between NH_4Cl and substrate concentrations (Figures 7). The non–elliptical nature of the contour plots depicts that there is no mutual interaction between the test variables. The experimental data was then analyzed statistically to obtain the following regression equation:

$$Y_{EA} = 0.5048 + 0.0707\ X_1 + 0.111\ X_2 - 0.1734\ X_3 - 0.2556\ X_1^2 + 0.4564\ X_2^2 + 0.1504\ X_3^2 - 0.0284\ X_1X_2 + 0.0227\ X_1 X_3 - 0.3951\ X_2\ X_3$$

Where, Y_{EA} – Response variable representing enzyme activity, X_1 – initial substrate concentration, X_2 – pH and X_3 – NH_4Cl concentration.

The determination coefficient value for this model equation was reasonably good under the experimental condition used in this study. The ANOVA result for the quadratic model is given in Table 3. In general the Fischer's variance ratio, the F value should be higher than the low probability, P values, for the predictions to be significant. This statistical analysis was done at the 95% confidence interval by the software, MINITAB 14. In this study among the main, squared and interaction effects of the variables on the enzyme activity, the squared effects played a major role for enzyme activity (F value of 7.61, P value of 0.006 than the main and interaction effects.

To understand the pattern of interaction and to envision synergistic and antagonistic effects between test variables, the student's t–test and P values were tabulated using the software as shown in Table 4. The larger magnitude of t value (either ±) and smaller P value, the more significant is the corresponding coefficient (Liu *et al.*, 2004). Student's t–test was employed to determine the knowledge of the error mean square that is essential in testing the significance of the estimated coefficient of the regression equation. The student's t–test value can be obtained by dividing each coefficient by its standard error. A large 't' value implies that the coefficient is much greater than its standard error. The squared effects of pH and substrate concentration (P – 0.003 and 0.055) had a major edge over other interaction and main effects. However the values of "t" and their sign imply the impact of their effects on the enzyme activity. The squared effects of pH increased the enzyme activity (t = 3.87), while the effects of substrate concentration (t value of –2.167) decreased the enzyme activity. On the other hand, the interaction effects between pH and NH_4Cl also showed negative effects on the enzyme activity with low P values (0.032). All the other coefficients (t values) were found to be insignificant. More precisely, the results from this study relied more on the square and interaction effects of the process variables than the main effects, while complex interactions were manifested with a statistical significance.

The optimum sets of operating conditions were obtained by solving the regression equation using the Monte Carlo simulation technique. The optimal values were first obtained in coded units (0.1523, 0.0934 and 0.6435) and then converted to the respective uncoded (real) units using the formulae described in Montgomery (1991). The optimal values of initial substrate concentration, pH and ammonium chloride concentration were 1.8066%, 4.193 and 0.5645% respectively. At these optimal conditions the enzyme activity was 2.388 U/ml, which was higher than the activity observed during regular experimentation. Nissen *et al.*, (1992) have suggested an optimal pH of 6–8 for increased production of xylanase. Horikoshi and Atsukawa (1973) were the first to report xylanase from *Bacillus sp.*, which was also active under high pH conditions. In this study, the model predicts the optimal concentration of xylanase production to be in lower pH range. Furthermore, the coded values were substituted in the regression equation to obtain the predicted enzyme activity, and a good correlation between the measured and predicted responses was noticed, as seen from their R^2 value (0.8950). The findings of the present investigation compare well with the work of Ellaiah *et al.*, (2002) on response surface optimization of the critical medium components for the production of alkaline protease by a newly isolated *Bacillus species.* Similarly, CCD techniques have been used to optimize maximum xylanase yields by *Schizophyllum commune* and *Thermomyces sp* with activity of 5.74 U/ml and 2.74 U/ml under submerged conditions (Haltrich *et al.*, 1993; Purkarthofer *et al.*, 1993). Senthil kumar *et al.*, (2005) optimized xylanase yield at 1024 U/gm of wheat bran using *Aspergillus fischeri* under solid state fermentation conditions. The optimum condition predicted by the model was verified by carrying experiments in triplicate using the same procedure outlined earlier to monitor the

enzyme activity. The model predicted enzyme activity value agreed well with the experimental result with an error of 7.6%.

4. Conclusions

This research work demonstrates that response surface methodology can be a powerful and simple tool to effectively analyze the results and to determine optimal conditions for xylanase production.

Laboratory scale batch experiments were performed with commercially available oat as the substrate in a mineral salt media under controlled conditions. The results from this study showed that under optimum values of substrate concentration: 1.8066 %, pH: 4.913 and NH_4Cl: 0.5645 %, the enzyme activity would be 2.388 U/ml. Further analysis with surface plots reveal that pairing factors produce saddle point response after a critical value indicating that some of the interactions were insignificant.

Acknowledgement

The authors of this paper would gratefully thank the Faculty of Engineering and Technology, Annamalai University, India for their continued support through research grants and Prof T. Swaminathan, Environmental Management Research Laboratory, Department of Chemical Engineering, Indian Institute of Technology Madras for his research suggestions.

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Table 1. Range and levels of process variables

Variable	Range and level				
	–∝	**–1**	**0**	**+1**	**+∝**
X_1, Substrate concentration	0.5227	1	1.7	2.4	2.8772
X_2, pH	1.8068	3	4.75	6.5	7.6931
X_3, NH_4Cl concentration	0.3318	0.4	0.5	0.6	0.6681

Table 2. Enzyme activity measured at different combinations of substrate concentration, pH and NH_4Cl concentration

Run No	**Oat** (%)	**pH**	**NH_4Cl** (%)	**Enzyme activity (U/ml) Measured**
1	1.000	3.000	0.400	0.333
2	2.400	3.000	0.400	0.280
3	1.000	6.500	0.400	1.253
4	2.400	6.500	0.400	1.788
5	1.000	3.000	0.600	0.131
6	2.400	3.000	0.600	0.869
7	1.000	6.500	0.600	0.172
8	2.400	6.500	0.600	0.096
9	0.523	4.750	0.500	0.175
10	2.877	4.750	0.500	0.070
11	1.700	1.807	0.500	2.190
12	1.700	7.693	0.500	2.082
13	1.700	4.750	0.332	1.267
14	1.700	4.750	0.668	1.274
15	1.700	4.750	0.500	0.486
16	1.700	4.750	0.500	0.480
17	1.700	4.750	0.500	0.484
18	1.700	4.750	0.500	0.490
19	1.700	4.750	0.500	0.481
20	1.700	4.750	0.500	0.492

Table 3. Anova for the quadratic regression model for enzyme activity

Source	**Degrees of Freedom**	**Seq Sum of square**	**Adj Sum of square**	**Adj Mean Square**	**F Value**	**P Value**
Regression	9	6.483	6.483	0.720		
Linear	3	0.649	0.649	0.216	3.59	0.029
Square	3	4.575	4.575	1.525	1.08	0.401
Interaction	3	1.259	1.259	0.419	7.61	0.006
Residual Error	10	2.004	2.004	0.2004	2.09	0.165
Lack–of–fit	5	0.0001	0.0001	0.400		
Pure error	5	8.488	8.488	0.00002		
Total	19					

Table 4. Significance test for main and interaction effects of the variable on enzyme activity measured under varying operating conditions

Independent variables (parameters)	Coefficient (β)	Standard error (β)	't'– value	P– value
Constant	0.5048	0.1826	2.765	0.020
X_1	0.0707	0.1211	0.584	0.572
X_2	0.1110	0.1211	0.916	0.381
X_3	–0.1738	0.1211	–1.435	0.182
X_1^2	–0.2556	0.1179	–2.167	0.055
X_2^2	0.4564	0.1179	3.870	0.003
X_3^2	0.1504	0.1179	1.275	0.231
X_1X_2	–0.0284	0.1583	–0.179	0.861
X_1X_3	0.0227	0.1583	0.143	0.889
X_2X_3	–0.3951	0.1583	–2.496	0.032

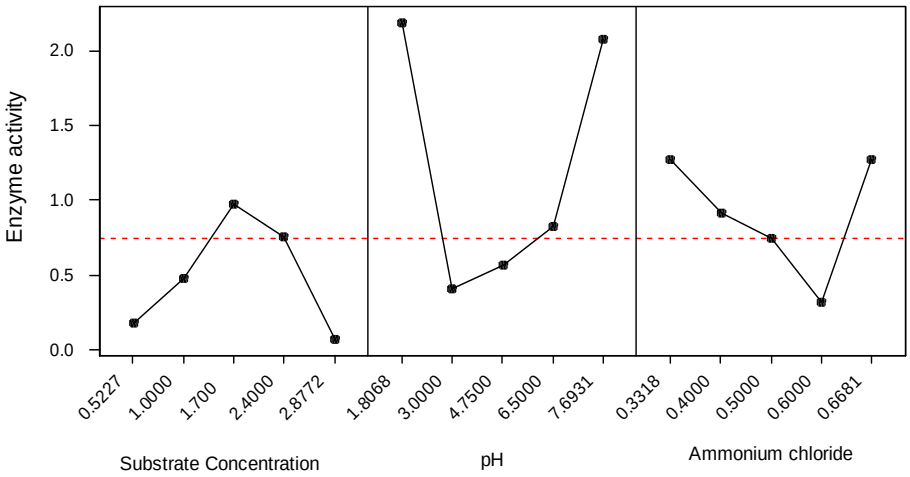

Figure 1. Main effects plot of process parameters on enzyme activity

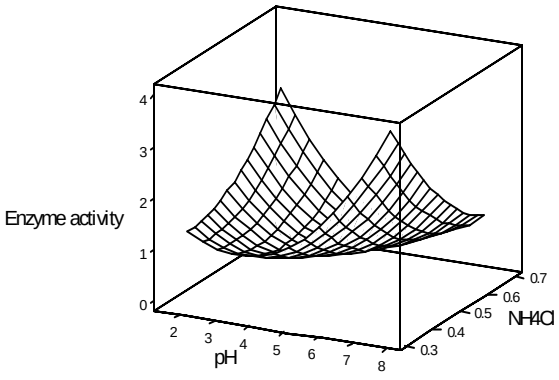

Figure 2. Response surface plot for enzyme activity at different ranges of pH and NH4Cl concentrations

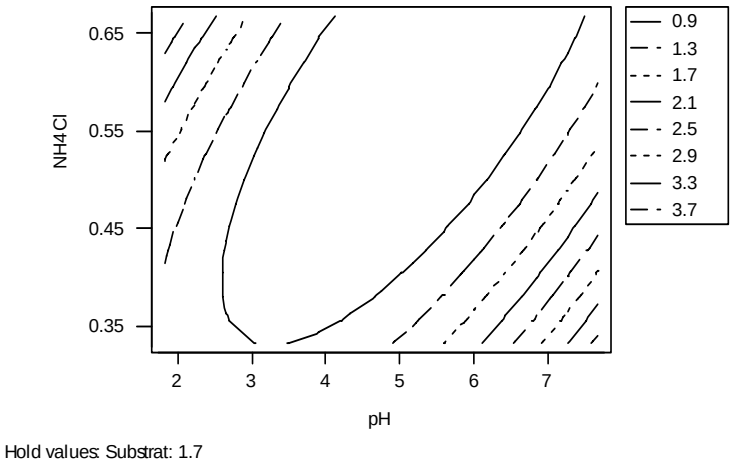

Figure 3. Contour plot for enzyme activity at different ranges of pH and NH4Cl concentrations

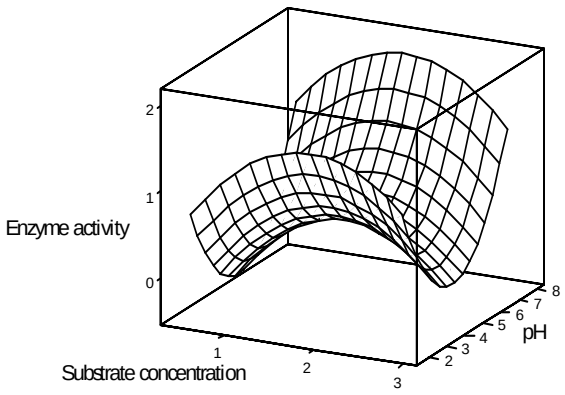

Figure 4. Response surface plot for enzyme activity at different ranges of substrate concentration and pH

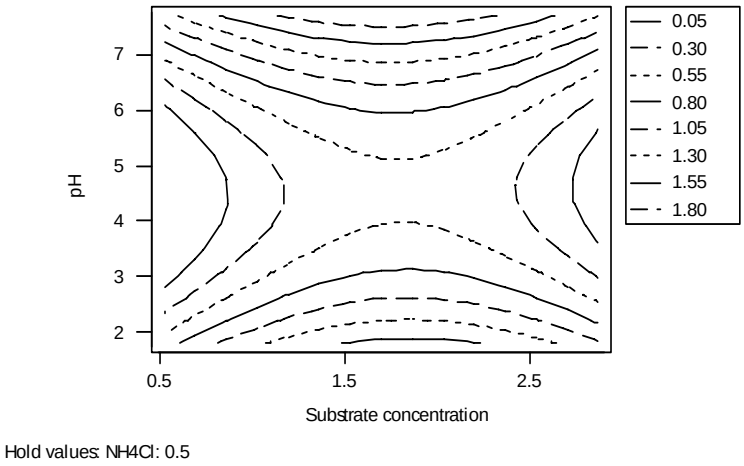

Figure 5. Contour plot for enzyme activity at different ranges of substrate concentration and pH

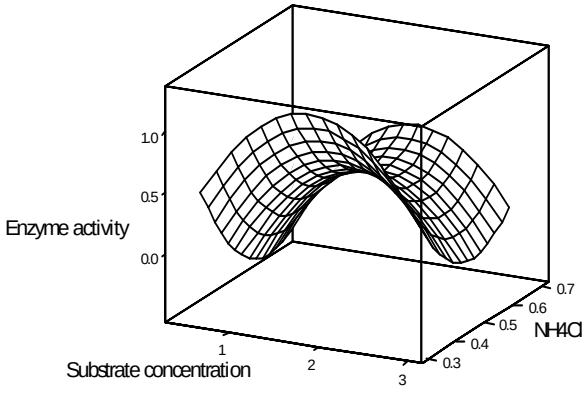

Figure 6. Response surface plot for enzyme activity at different ranges of substrate concentration and NH4Cl concentrations

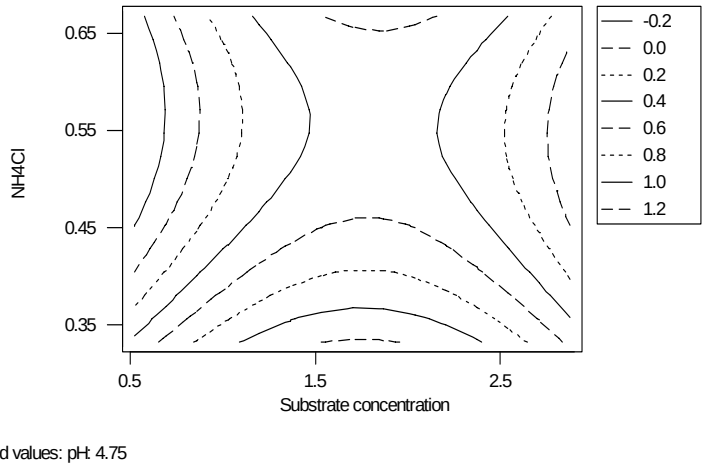

Figure 7. Contour plot for enzyme activity at different ranges of substrate concentration and NH4Cl concentrations

Journal of Agricultural Science
http://www.ccsenet.org/jas
Vol. 2, No. 1
March 2010

Genotype-Environment Interaction and Genetic Parameters in Chickpea (*Cicer arietinum* L.) Landraces

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The research is financed by General Commission for Scientific Agricultural Research (GCSAR) and International Center for Agricultural Research in the Dry Areas (ICARDA). Syria.

Abstract

In order to determine the desirable line for chickpea rainfed sowing, seven local lines (K- 6, G- 35, D- 15, F-20, B -17, H- 45 and M- 20) were selected from Syrian chickpea landraces. These lines were grown during two seasons 2006 / 2007- 2007 / 2008 at two different locations (Tal-sandal: with a mean annual precipitation of 475mm, Harran: with a mean annual precipitation of only 300 mm). Genotype-environment interaction and genetic parameter were studied for seed yield per plant /g, days to maturity and protein content.

The results showed that effect of location (L) and season (E) was highly significant ($P < 0.01$) while the interaction among Locations, Seasons and Genotypes (L x E x G) was not significant ($P > 0.05$).

The heritability for protein content varied from (0.83) to (0.93) that indicated the presence of a considerable proportion of total variability due to genetic causes. A high genetic advance (GA) (51.5 to 62.7) % was achieved for seeds yield per plant. The environmental variance (σ2e) was very low for days to maturity and protein content. The differences between genotypic (GCV) and phenotypic (PCV) coefficient of variability were very small.

The results recommended to selection line M-20 that was the desirable line for both seed yield per plant (33 to 40) g and protein content (22.17 to 24.72%) as compared to other lines.

Keywords: Chickpea, Genotype-Environment, Genetic parameters, Protein content

1. Introduction

Chickpea is the second most cultivated grain legume in the world after phaseolus bean (Rubio *et al.,* 1998; Rubio *et l.,* 2004). Chickpea is traditionally grown as a rainfed spring crop in Syria, mainly on the soil moisture conserved during winter rains in areas with seasonal precipitation of about 300mm. Winter chickpea sowing produces correspondent plants with a longer flowering period and higher yield than those sown in spring. All winter improved varieties (Ghab3- Ghab4- Ghab5) which released by international Center for Agricultural Research in the Dry Areas (ICARDA) were susceptible to the developed fierce lines of Ascochyta blight.

In recent years, Ascochyta blight has caused widespread yield losses in chickpea (Knights and Siddique, 2002), it can spread in epidemic form and results in 75 to 100% yield loss for the winter improved varieties. Chickpea farmers in Syria still keep and cultivate their own seeds generation to avoided winter sowing because of the risk of heavy crop loss.

Syria's flora includes several local cultivars and landraces of chickpea are found in diverse forms in many zone of Syria.

Chickpea landraces are potentially a useful germplasm resource of genetic variability for traits of interest such as stress resistance and tolerance and grain quality characteristics (Moulla *et al.,* 2005).

Several researchers (Ghafoor *et al.,* 2000; Malik *et al.,* 1988) have emphasized the utility of the estimates of Heritability (H2) and genetic advance (GA) in the prediction of response of quantitative characters to selection in chickpea. Heritability alone is not very useful but this statistic along with genetic advance is valuable (Johnson *et al*., 1955).

Genotype x environment interaction (G x E) is increasingly important, because breeding programs tend to be more internationally oriented. During recent decades, new improvements have been accomplished in plant physiology, agronomy, and statistics and some incorporated approaches emerged for G x E interactions evaluation (Brancourt, 1999).

Presence of genotype × environment interaction necessitates evaluation of genotypes in a wide range of environments to find desirable genotypes (Zali *et al.,* 2008).

The major goal of this work to study the genotype-environment interaction and some genetic parameter for these selected lines. Moreover, selection the favorite promise line that had good qualitative and quantitative characteristics under rainfed spring sowing.

2. Materials and Methods

2.1 Plant Material and agricultural practices

This research achieved in the General Commission for Scientific Agricultural Research (GCSAR) in Syria and International Center for Agricultural Research in the Dry Areas (ICARDA).

Seven local lines (K-6, G-35, D-15, F-20, B-17, H- 45 and M-20) have been selected from Syrian chickpea landraces (these landraces collected by GCSAR and ICARDA) (Table 1).

These lines sown in a Randomized Complete Block Design (RCBD) with three replications during two seasons / 2007 2006-2007 / 2008 in two different locations (Tal-sandal: with a mean annual precipitation of 475mm, Harran: with a mean annual precipitation of only 300 mm). Seeds were hand sown on 15 March for both 2006 / 2007 - 2007 / 2008 seasons. Each line sown in 2 rows of 5 m length, spaced 45 cm between rows and 35 cm between plants.

Plots were fertilized with 100 kg / ha super phosphate (P2O5: 46% P). The experiment was conducted under rainfed condition. Hand weeding and pesticide application..

After maturity, ten plants were apart harvested from each plot. Data were recorded on seeds yield per plant (gm) and days to maturity. Protein content was estimated according to Kjeldahl method.

2.2 Genetic Parameters Estimates

Heritability in broad sense (H^2 or h^2) was estimated according (Falconer, 1989) as equation (1):

$$h^2 = \frac{\sigma^2_g}{\sigma^2_p} \quad (1)$$

h^2: Heritability; $\sigma^2 g$: genotypic variance and $\sigma^2 p$: phenotypic variance. Genotypic ($\sigma^2 g$), Phenotypic ($\sigma^2 p$) and Environmental Variances ($\sigma 2e$) were obtained from the analysis of variance table according (Comstock and Robinson, 1952) as equations (2):

$$\sigma^2 g = (MS2-MS3)/r;\ \sigma^2 p = MS2/r \text{ and } \sigma 2e = MS3. \quad (2)$$

(Where r: replication, MS2: Mean square for cultivar, MS3: Mean square for error).

Coefficient of Variability (CV %); Genotypic Coefficient of Variability (GCV%); Environmental Coefficient of Variability (ECV%) and Phenotypic Coefficient of Variability (PCV%) were calculated as suggested by Burton (1952) as equations (3):

$$CV = \sigma ph / X * 100;\ G.C.V = \sigma^2 g / X * 100;\ PCV = \sigma^2 p / X * 100 \text{ and } \sigma 2e = \sigma^2 g / X * 100.$$

X: Grand Mean. (3)

Genetic advance (GA) was calculated with the method suggested by (Singh and Chaudhary 1979; Allard, 1960) as equations (4):

$$GA = k.\sigma ph.h^2 \quad (4)$$

Where: σph : Standard deviation; K:(constant = 2.06 at 5%selection intensity) and GA%: genetic advance in percentage mean.

Analysis of variance (ANOVA), Genotype-environment interaction (L x E x G), Standard Error (SE ±) and Least Significant Difference L.S.D at P ≤ 1%, 5% performed using a computer software (Genstat 7th edition and SPSS 15).

3. Results and discussion

We will demonstrate the results in the locations and seasons for each studied characteristic and genetic parameters separately follow as:

3.1 Mean of Seeds Yield per Plant: (SY/P)

The maximum seeds yield per plant (SY/P) was recorded for line M- 20 in both locations and seasons. The line M- 20 had (40) g in Tal Sandal and (36) g in Harran location for 2006/2007, also M- 20 had (37) g, (33) g in Tal Sandal and Harran respectively in 2007 / 2008 seasons.

The effect of location (L) and season (E) was significant (P < 0.01) while the interaction (L x Ex G) was not significant **(** P > 0.05) **(**Table 2).

3.2 Mean of Days to Maturity: (DM)

Days to maturity (DM) in 2006 / 2007 varied from (65.33) for Fo-20 in Harran to (80.67) days for D-15 in Tal-Sandal while varied from (64.67) days for Fo-20 in Harran to (77.33) days for D-15 in Tal-Sandal at 2007 /2008 season. The Analysis of Variance (ANOVA) showed significant differences for day to maturity (P < 0.01) in both locations and seasons.

The interaction of location (L), season (E) and (Lx G) was significant at (0.01) level of probability. The interaction (L x E x G) was not significant (P > 0.05).(table 3).

3.3 Mean of Protein Content %: (PC)%

The Analysis of Variance (ANOVA) showed significant differences for protein content (PC) % in both locations and seasons (P < 0.01). The effect of location (L), season (E) and line (G x L) interaction was significant at (0.01) level of probability while the interaction (E x G), (L x E) and (L x G x E) was not significant P > 0.05 (Table 4).

3.4 Heritability and genetic parameters

Estimates of heritability for seeds yield per plant varied from (0.96) to (0.99) whereas days to maturity gave the highest estimate of heritability (0.99) in both locations and seasons, which indicated that total variability was due to genetic causes.

A high estimate of heritability for protein content varied from (0.83) to (0.93) reflected that selection could be effective for improving the trait and the environmental influence for protein content was very low.

The genetic advance (GA) % was the highest for seeds yield per plant (varied from 51.50% to 62.7%).

The environmental variance **(**σ2e) was very low for days to maturity and protein content compared to environmental variance **(**σ2e) for seeds yield per plant.

The differences between genotypic coefficient (GCV) and phenotypic coefficient (PCV) of variability were very small for all studied characteristics indicating negligible role of environment (table 5).

The interaction (L x E x G) for all studied characteristics was not significant, probably due to high adaptation for these landraces with climatic conditions under many zones (locations) along years (seasons), for thousands of year's chickpea landraces have evolved under the influence of natural and artificial selection as performed by many generations of farmers.

Abdel *et al.,* (2005) found no significant interaction detected between seasons; the main source of yield variation in the Mediterranean region is variation in rainfall.

Tuba *et al.,* (2004) Reported that Irrigation (E) X Cultivars (G) interaction was significant for some characterictes in some cultivares, this revealed the different response of these cultivares under rainfed and irrigation conditions.

Zvereny *et al.,* (2006) found that heritability for most Characters and Genotype x Year interaction Variance (GYV) were small (heritability ranged from 5.47% to 51.66%) due to larger phenotypic variances, indicating environmental influence.

These results are in agreement with Jahagirdar *et al.* (1994) who found high heritability together with high genetic advance for 100 seed weight and seeds yield per plant.

Arshad *et al.* (2004) found high rang for seeds yield per plant (11.7) for twenty-four candidate varieties of chickpea.

High heritability estimates were reported by Tripathi (1998); Kumar *et al*. (1999) and Saleem *et al.,* (2002) for 100-seed weight (93.6%).

The results are in contrast with Kumar and Krishna (1998) who found that grain yield per plant had poor heritability estimates.

4. Conclusions

The available information's from our study will be helpful to devise an efficient selection criterion to select the most desirable lines under rainfed conditions in Syria.

The results suggested that improvement for seeds yield per plant can be efficient through selection the line M-20 that had highest seed yield per plant (33 to 40)g and high protein content (22.17 to 24.72%) as compared to other lines.

We concluded there is significant differences among studied lines, also the effect of location (L) and season (E) was significant ($P< 0.01$) suggested that lines behaved differentially in each location and season. The interaction (L x E x G) was no significant for all characteristics ($P > 0.05$).

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Table 1. List of (7) chickpea accessions used in study

Entry No.	Line Name	Source
1	K- 6	Gene Bank of GCSAR, Syria
2	G- 35	Gene Bank of GCSAR, Syria
3	D- 15	Gene Bank of GCSAR, Syria
4	F-20	Gene Bank of GCSAR, Syria
5	B -17	Gene Bank of ICARDA, Syria
6	H- 45	Gene Bank of GCSAR, Syria
7	M- 20	Gene Bank of GCSAR, Syria

Table 2. ANOVA and Interaction for seeds yield per plant in (7) chickpea selected lines grown during two seasons 2006 / 2007- 2007 / 2008 at tow locations Tal-Sandal and Harran

Locations (L) / Lines (G)		Seasons (E)			
		2006 / 2007		2007 / 2008	
		Tal-Sandal (SY/P)	Harran (SY/P)	Tal-Sandal (SY/P)	Harran (SY/P)
K- 6		35.00	30.00	29.00	27.00
G- 35		29.00	25.00	24.33	22.33
D- 15		20.00	16.33	17.67	13.67
F-20		23.00	21.00	20.00	19.00
B -17		21.67	16.67	19.33	14.67
H- 45		33.00	30.00	30.00	28.00
M- 20		40.00	36.00	37.33	33.00
Grand Mean (X)		28.81	25.00	25.38	22.50
ANOVA	Mean Square	171.762	165.778	151.714	156.206
	F- Value	32.205	39.116	33.894	99.404
	P -Value	0.000**	0.000**	0.000**	0.003**
$LSD_{0.01}$		5.613	5.004	5.142	3.047
$LSD_{0.05}$		4.0444	3.605	3.705	4.044
SE ±		1.622	1.584	1.522	1.50
CV%		25.8	29.04	27.50	30.70
Interaction	G	0.000 < 0.01**			
	L	0.000 < 0.01**			
	E	0.001 < 0.01**			
	L x G	0.387 > 0.05 n.s			
	E x G	0.719 > 0.05 n.s			
	L x E	0.389 > 0.05 n.s			
	L x E x G	0.916 > 0.05 n.s			

, * Significant at 0.05 and 0.01 percent probability level, respectively. n.s: not Significant.

Table 3. ANOVA and Interaction for days to maturity in (7) chickpea selected lines grown during two seasons 2006 / 2007- 2007 / 2008 at tow locations Tal-Sandal and Harran

Locations (L) / Lines (G)		Seasons (E)			
		2006 / 2007		2007 / 2008	
		Tal-Sandal (DM)	Harran (DM)	Tal-Sandal (DM)	Harran (DM)
K- 6		70.33	67.33	69.33	65.67
G- 35		69.33	66.67	68.33	65.67
D- 15		80.67	77.33	77.33	76.33
F-20		70.00	65.33	69.00	64.67
B -17		71.67	68.67	70.33	67.00
H- 45		73.00	69.33	71.33	68.33
M- 20		75.33	70.67	73.67	69.67
Grand Mean (X)		72.90	69.33	71.33	68.19
ANOVA	Mean square	47.746	46.667	30.444	166.389
	F- Value	125.333	140.0	71.037	47.54
	P- Value	0.000**	0.000**	0.000**	0.000**
$LSD_{0.01}$		1.500	1.403	1.591	1.299
$LSD_{0.05}$		1.081	1.011	1.146	0.936
SE ±		0.830	0.823	0.670	0.830
CV%		5.60	5.44	4.31	5.58
Interaction	G	0.000 < 0.01**			
	L	0.000 < 0.01**			
	E	0.001 < 0.01**			
	L x G	0.001 < 0.01**			
	E x G	0.128 > 0.05 n.s			
	L x E	0.246> 0.05 n.s			
	L x E x G	0.049 < 0.05 *			

Table 4. ANOVA and Interaction for protein content in (7) chickpea selected lines grown during two seasons 2006 / 2007- 2007 / 2008 at tow locations Tal-Sandal and Harran

Locations (L) / Lines (G)		Seasons (E)			
		2006 / 2007		2007 / 2008	
		Tal-Sandal (PC)%	Harran (PC)%	Tal-Sandal (PC)%	Harran (PC)%
K- 6		22.07	21.45	21.40	20.93
G- 35		22.79	23.02	22.10	22.20
D- 15		23.08	21.49	22.43	21.00
F-20		21.15	21.09	20.63	20.40
B -17		21.19	20.54	20.63	20.00
H- 45		21.17	21.62	20.53	21.03
M- 20		24.72	22.58	23.87	22.17
Grand Mean (X)		22.31	21.69	21.66	21.10
ANOVA	Mean square	5.299	2.169	4.553	2.045
	F- Value	13.618	5.872	9.22	6.065
	P	0.000**	0.003**	0.000**	0.003**
$LSD_{0.01}$		1.516	1.477	1.708	1.411
$_{0.05}$		1.092	1.064	1.231	1.017
SE ±		0.298	0.208	0.286	0.200
CV%		6.1	4.4	6.0	4.4
Interaction	G	0.000 < 0.01**			
	L	0.000 < 0.01**			
	E	0.000 < 0.01**			
	L x G	0.000 < 0.01**			
	E x G	0.946 > 0.05 $^{n.s}$			
	L x E	0.369 > 0.05 $^{n.s}$			
	L x E x G	0.677 > 0.05 $^{n.s}$			

Table 5. Genetic parameters for some characteristics in (7) chickpea selected lines grown during two seasons 2006 / 2007- 2007 / 2008 at tow locations Tal-Sandal and Harran

Trait	Season (E)	Location (L)	σ2g	GCV	σ2e	ECV	σ2p	PCV	H2	GA	GA%
SY/P	2006 / 2007	Tal - sandal	55.48	192.5	1.78	6.170	57.25	198.7	0.96	14.84	51.50
		Harran	53.85	215.3	4.24	16.95	55.26	221.0	0.97	14.57	58.29
	2007 / 2008	Tal - sandal	49.08	193.4	4.48	17.64	50.57	199.3	0.97	13.94	54.94
		Harran	51.5	228.8	1.57	6.97	52.1	231.2	0.99	14.12	62.70
DM	2006 / 2007	Tal - sandal	15.79	21.66	0.381	0.523	15.92	21.83	0.99	7.81	10.71
		Harran	15.44	22.28	0.333	0.480	15.56	22.44	0.99	7.72	11.13
	2007 / 2008	Tal - sandal	10.01	14.03	0.429	0.601	10.15	14.23	0.99	6.24	8.75
		Harran	15.75	23.10	0.286	0.419	15.85	23.24	0.99	7.79	11.42
PC%	2006 / 2007	Tal - sandal	1.64	7.34	0.39	1.74	1.77	7.92	0.93	2.60	11.67
		Harran	0.60	2.8	0.37	1.70	0.72	3.3	0.83	1.63	7.52
	2007 / 2008	Tal - sandal	1.35	6.25	0.49	2.28	1.52	7.01	0.89	2.40	11.09
		Harran	0.57	2.70	0.34	1.60	0.68	3.23	0.84	1.59	7.51

Journal of Agricultural Science
http://www.ccsenet.org/jas
Vol. 2, No. 1
March 2010

An Empirical Simplification of the Temperature Penman-Monteith Model for the Tropics

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Abstract

A simple empirical equation (EPM) is presented to considerably shorten the computational steps required to estimate reference grass evapotranspiration (ETo) in the tropics using the FAO-56 Penman-Monteith equation (TPM) when the only available weather data are those of temperature. Generally EPM predicted TPM ETo with very high efficiencies, achieving statistical performance measures as high as $r^2 = 1.00$, $E_1 = 0.98$, $E_2 = 1.00$, MAE=0.01 mm/day in tests on data from six locations in four countries in West Africa. EPM was of general form $ET_{o,EPM} = T^b R_s/k - R_a^{\chi}/17000$, where ET_o = reference grass ETo (MJ $m^{-2} d^{-1}$), T = daily average air temperature ($^{\circ}$C), R_s = estimated solar radiation (MJ $m^{-2} d^{-1}$), R_a = computed extraterrestrial radiation (MJ $m^{-2} d^{-1}$); b, k, and χ, were parameters computed from local latitude and temperature data. The simplicity of EPM is expected to encourage wider usage of TPM ETo estimates which are more accurate than estimates obtained by using locally-uncalibrated versions of simpler ETo models where only temperature data are available.

Keywords: Evapotranspiration, FAO-56 Penman-Monteith, Coefficient of efficiency, ETo, Water-use efficiency, Empirical, Reference grass ETo

1. Introduction

Reference evapotranspiration (ETo) estimates are very important in irrigation system design and operations. ETo estimates are used to estimate crop evapotranspiration (ETc) rates which are used to determine design peak irrigation system capacities and also irrigation water requirements and irrigation schedules (Keller & Bliesner, 1990). Because ETc is difficult and expensive to measure directly, it is usually conveniently estimated indirectly from mathematical models using climatic data inputs (Farahani *et al.*, 2007).

Although the most widely accepted mathematical model for reference grass ETo estimation from meteorological data is the FAO-56 Penman-Monteith equation (PM) (Allen *et al.*, 1998), it requires data, apart from temperature data, that are normally measured at few weather stations, even in the developed countries. For some locations the required climate data are available but of questionable quality, especially in developing countries (Droogers & Allen, 2002). However, as a minimum, many weather stations around the world, even in developing countries, collect temperature data of acceptable quality (Hargreaves & Allen, 2003). Therefore ETo models that require only temperature data, but are highly accurate, are very useful in data-poor areas of the world.

Where only temperature data are available, the FAO recommends using the temperature-only Penman-Monteith (TPM) version of PM, whereby all the unavailable data are estimated according to certain outlined procedures (Allen *et al.*, 1998). The TPM has been reported to give reliable estimates in several locations around the world (Campbell Scientific, 1998; Jabloun & Sahli, 2008; Popova *et al.*, 2006). Two other temperature ETo models are the Hargreaves & Samani (1985) (HG) and the Turc (1961) (TU) models which have been reported to give reliable ETo estimates (Lu *et al.*, 2005; Yoder *et al.*, 2005; Hargreaves & Allen, 2003), but only after proper local calibration (Gavilan *et al.*, 2006). However because of lack of equipment for proper local calibration of TUand HG, the attractive simplicity of TU and HG, and discouraging complexity of TPM, uncalibrated HG and TU are often used in practice, especially in the developing world.

The goal of this study was to considerably simplify the computation of TPM ETo estimates with little loss of accuracy. The main objective was to develop one single and simpler equation thatwould give essentially the same ETo estimates

as TPM, but that would be as easy to use as simple temperature-based models such as TU and HG. The single equation, named the Empirical Penman-Monteith equation (EPM), should be applicable to data from other weather stations in West Africa apart from the original development site. EPM should use the same weather data as TPM, and should eliminate more than 10 of the intermediate computations required to apply TPM.

2. Materials and methods

The empirical Penman-Monteith equation (EPM) for the temperature Penman-Monteith equation (TPM) was developed using daily temperature data from six weather stations located in four countries in West Africa. The weather and other required data for the Accra site were obtained from the Water Research Institute (WRI) in Accra, while that for the other sites were obtained from TuTiempo (2008) and then processed for spreadsheet use. Where average wind speed data was not available the global average of 2 m/s reported in Droogers & Allen (2002) was assumed.

All the computations were executed in Microsoft Excel XP spreadsheet installed on Microsoft Windows XP operating system on an HP Pavilion dv6000 laptop computer with Intel (R) Core (TM)2 CPU T7200 2.00 GHz, 1.00 GB RAM.

EPM was developed by starting with a simple form of EPM, and then computing two sets of daily reference grass evapotranspiration (ETo) values (one by TPM and the other by EPM), and then tweaking the EPM parameter values using a numerical algorithm to make the EPM results as close as possible to those of TPM. Examination of the temporal plot of the errors informed the addition of other terms to EPM during the development process. Details of the forms of the TPM and EPM equations and the computational procedures are given in the sections that follow.

2.1 Computation of daily EPM and TPM ETo

The following parameters were computed for every day of the year from the daily temperature data for each weather station using the indicated equations:

1. Average temperature, T, using Eqn. (14);
2. Slope of the saturation vapor pressure versus temperature curve, Δ, using Eqn. (13);
3. Latent heat of vaporization of water, λ, using Eqn. (16);
4. Psychrometric constant, γ, using Eqn. (15);
5. Actual vapor pressure, e_a, using Eqn. (2);
6. Saturation vapor pressure, e_s, using Eqn. (3);
7. Extraterrestrial radiation, R_a, using Eqn. (7);
8. Solar radiation, R_s, using Eqn. (6);
9. Clear sky radiation, R_{so}, using Eqn. (12);
10. Net longwave radiation, R_{nl}, using Eqn. (11);
11. Net shortwave radiation, R_{ns}, using Eqn. (6);
12. Net radiation, R_n, using Eqn. (4);
13. TPM reference grass evapotranspiration, $ET_{o,TPM}$, using Eqn. (1);
14. EPM reference grass evapotranspiration, $ET_{o,EPM}$, using the developed EPM equation, Eqn. (19), with guessed initial values for the unknown parameters of the EPM equation.

2.2 Development of the form of EPM

After computing the daily values of the parameters in steps 1-14 above, an optimal form of EPM and its optimal parameter values were determined following these steps:

1. The modified coefficient of efficiency, E_1, for $ET_{o,EPM}$ versus $ET_{o,TPM}$ for the annual set of data was computed using Eqn. (18);
2. The solver function in Microsoft Excel was set up to maximize E_1 by numerically varying the values of the parameters of EPM at each stage of the development. The constraints were $s = 1$, and $c = 0$ where s, and c are respectively, the slope and intercept of the straight line of the plot of $ET_{o,EPM}$ versus $ET_{o,TPM}$;
3. The optimum values of the EPM parameters were the ones returned by the Microsoft Excel solver;
4. The residual, $ET_{o,TPM} - ET_{o,EPM}$, versus time was plotted and modeled as an additional term to improve the efficiency of the current form of EPM;
5. A new value of E_1 was computed and a new set of optimal parameter values was numerically determined using the

solver in Microsoft Excel to maximize the new E_1;

6. Other simple forms of EPM were experimented with and the selected optimal form of EPM was that which yielded the highest E_1 values.

2.3 Calibration and tests of EPM

After the development of its form using the Accra data, EPM was calibrated for each of the other tropical sites using the following steps:

1. $ET_{o,TPM}$ and $ET_{o,EPM}$ were computed for each day of one year of average daily data;

2. The optimal values of the parameters of EPM were obtained from the numerical solver by optimizing E_1.

3. The calibrated EPM was tested by using it to compute daily $ET_{o,EPM}$ from fresh data for each site; for each site (except Accra) the 1999-2003 data were used for EPM development while the 2004-2008 were used for testing. For the Accra site the average 1998-2006 data were used for EPM development while 2007 data were used for testing.

4. The statistical performance parameters E_1, E_2, MAE, r^2, m, and c were computed for the generated $ET_{o,TPM}$ versus $ET_{o,EPM}$ data and compared to their ideal values.

2.4 FAO-56 Penman-Monteith equation (PM)

The FAO-56 Penman-Monteith (PM) for evapotranspiration estimation from weather data is

$$ET_{o,PM} = \frac{0.408\Delta(R_n - G) + \gamma \dfrac{900}{(T+273)} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)} \tag{1}$$

where, $ET_{o,PM}$ = reference grass evapotranspiration (mm d^{-1}); R_n = net radiation at the crop surface (MJ m^{-2} d^{-1}); G = soil heat flux density (MJ m^{-2} d^{-1}); T = mean daily air temperature at 2 m above ground (^{o} C); u_2 = wind speed at 2 m above ground surface (m s^{-1}); e_s = saturation vapor pressure (kPa), e_a = actual va por pressure (kPa), Δ = slope of vapor pressure-temperature curve (kPa $^\circ$ C^{-1}), γ = psychometric constant (kPa $^\circ$ C^{-1}) Allen *et al.* (1998). When all the data required to use Eq. 1 is unavailable it can still be applied by using various formulas to estimate the missing data from temperature data; this method of applying PM from only temperature data is called the temperature PM method (TPM) in this study, for which $ET_{o,PM}$ is replaced by $ET_{o,TPM}$ in Eqn. (1).

2.5 Temperature Penman-Monteith application (TPM)

TPM was used to estimate daily ETo from the maximum and minimum temperature data following the recommendations of Allen *et al.*, (1998) and (Allen, 2002). In the TPM the daily e_s, e_a, R_n, R_s, R_{ns}, R_{nl}, R_a, Δ, γ, P, and G data were all estimated from maximum and minimum temperatures T_m and T_n, respectively, elevation of the site above sea level (z) latitude of the location (ϕ), and the Julian day (J), using mathematical expressions.

The actual vapor pressure, e_a (kPa), was estimated by

$$e_a \approx 0.6108 \exp\left(\frac{17.27T_n}{T_n + 237.3}\right) \tag{2}$$

and saturated vapor pressure, e_s (kPa), was estimated by

$$e_s \approx \frac{0.6108}{2}\left[\exp\left(\frac{17.27T_x}{T_x + 237.3}\right) + \exp\left(\frac{17.27T_n}{T_n + 237.3}\right)\right] \tag{3}$$

The net radiation, R_n ($MJ\ m^{-2}\ d^{-1}$), was estimated as

$$R_n = R_{ns} - R_{nl} \tag{4}$$

where

$$R_{ns} \approx 0.77 R_s \tag{5}$$

and solar radiation, R_s ($MJ\ m^{-2}\ d^{-1}$), was estimated using

$$R_s = k_{Rs} \cdot R_a (T_x - T_n)^{0.5} \tag{6}$$

with k_{Rs} set to 0.19 for locations near large water bodies, and 0.16 for other locations (Hargreaves & Allen, 2003). R_a , extraterrestrial radiation ($MJ\ m^{-2}\ d^{-1}$) was computed from (Duffie & Beckman, 1991)

$$R_a = \frac{24 \times 60}{\pi} G_{sc} d_r [\omega_s \sin(\phi) \sin(\delta) + \cos(\phi) \cos(\delta) \sin(\omega_s)] \tag{7}$$

$$\delta = 0.4093 \sin\left(\frac{2\pi(284 + J)}{365}\right) \tag{8}$$

$$d_r = 1 + 0.033 \cos\left(\frac{2\pi J}{365}\right) \tag{9}$$

$$\omega_s = \cos^{-1}(-\tan(\phi) \tan(\delta)) \tag{10}$$

where G_{sc} = global solar constant ($0.0820\ MJ\ m^{-2}\ min^{-1}$); J = day of the year (Jan 1 = 1); d_r = relative earth-sun distance; ω_s = sunset hour angle (radians); ϕ = latitude (radians); δ = solar declination (radians).

The net long-wave radiation, R_{nl} ($MJ\ m^{-2}\ d^{-1}$), was estimated from

$$R_{nl} \approx \sigma \frac{(T_x + 273.16)^4 + (273.16 + T_n)^4}{2} \left(0.34 - 0.14\sqrt{e_a}\right)\left(\frac{1.35 R_s}{R_{so}} - 0.35\right) \tag{11}$$

where the clear sky solar radiation, R_{so} ($MJ\ m^{-2}\ d^{-1}$), was estimated using

$$R_{so} = (0.75 + 2 \times 10^{-5} z) R_a \tag{12}$$

and z = altitude above sea level (m). Δ was computed from

$$\Delta = 0.2(0.00738T + 0.8072)^7 - 0.000116 \tag{13}$$

where the average temperature, T , was calculated as

$$T = \frac{T_n + T_x}{2} \tag{14}$$

The psychrometric constant, γ , was calculated from

$$\gamma = \frac{c_p P}{\varepsilon \cdot \lambda} \tag{15}$$

where P = atmospheric pressure (kPa), c_p = the specific heat at constant pressure, ($MJ\ kg^{-1}\ K^{-1}$), ε = ratio of the molecular weights of water vapor/dry air, and the latent heat of water, λ ($MJ\ kg^{-1}$) was computed as

$$\lambda = 2.501 - 0.002361T \tag{16}$$

and P at elevation, z m above sea level, was estimated using (Allen, 2002)

$$P = 101.3\left[\frac{T + 273 - 0.0065z}{T + 273}\right]^{5.26} \tag{17}$$

Following normal practice for fully vegetated reference grass surface, the approximation $G \approx 0$ was used in this study (Pereira & Allen, 1999; DehghaniSanij *et al.*, 2004; Jabloun & Sahli, 2008; Allen *et al.*, 1998; Allen, 2002)

2.6 Statistical performance measures

The main measure of the ability of EPM to predict TPM ETo values was the modofied coefficient of efficiency, E_1, which is related to, but more discriminatory than the coefficient of efficiency, E_2 (Hall, 2001); In this paper they were defined generallly as E_c, Bardsley(Bardsley & Purdie, 2007; Legates & McCabe, 1999)

$$E_c = 1 - \frac{\sum_{i=1}^{N}\left|ET_{o,TPM}^{i} - ET_{o,EPM}^{i}\right|^{c}}{\sum_{i=1}^{N}\left|ET_{o,TPM}^{i} - \overline{ET_{o,TPM}^{i}}\right|^{c}} \tag{18}$$

where c is a positive integer, equal to 1 for E_1 and 2, for E_2. E_c ranges from $-\infty$ to 1.00, with higher values indicating better agreement between model EPM and TPM. If $E_c = 0$ then the mean of the TPM estimates, $\overline{ET_{o,TPM}^{i}}$, is as good an estimator as model EPM. For further information of the relative merits of E_1, E_2, and the coefficient of determination, r^2, as measures of model performance the reader is referred to Legates & McCabe (1999); Yoder *et al.* (2005); Zhang *et al.* (2008); Stöckle *et al.* (2004). The other measures of the performance of EPM were coefficient of determination (r^2), intercept of EPM versus TPM regression line (c), slope of the EPM versus TPM regression line (m), and annual mean absolute error (MAE).

3. Results and Discussion

3.1 General and specific EPM equations

The general form of the developed EPM equation was

$$ET_{o,EPM} = EPMp - EPMc = \frac{T^{b} R_s}{k} - \frac{R_a^{\chi}}{17000} \tag{19}$$

where $ET_{o,EPM}$ = reference grass evapotranspiration (MJ $m^{-2} d^{-1}$) with k, b, and χ being locally calibrated constants and all other symbols as previously defined. $ET_{o,EPM}$ in mm d^{-1} was obtained by dividing $ET_{o,EPM}$ (MJ $m^{-2} d^{-1}$) by the latent heat of vaporization of water, λ (MJ kg^{-1}). The calibrated form of eqn. (19) for the WRI site was

$$ET_{o,EPM} = \frac{T^{0.63} R_s}{14} - \frac{R_a^{2.54}}{17000} \tag{20}$$

The optimal parameter values for the six sites during calibration and testing are shown in Table 1

3.2 Minimizing EPM residuals

The final form of EPM consisted of two parts, the main part, EPMp, and the minor part, EPMc (see eqn. (19)). The purpose of EPMp was to predict ETo by a very simple mathematical expression while that of EPMc was to minimize the prediction errors (i.e., $ET_{o,EPM} - ET_{o,TPM}$) of EPM by modeling the errors of EPMp (i.e., $ET_{o,EPMp} - ET_{o,TPM}$). The residuals of EPMp when EPM=EPMp were less precise and of a less definite form (Fig. 1a) than when EPM=EPMp+EPMc (Fig. 1b).

The improvement in the $ET_{o,TPM}$ prediction efficiency of EPM by the introduction of EPMc was reflected in the reduced scatter and fluctuation in the temporal residual plots. For example when EPM=EPMp was calibrated with the

average daily 1998-2006 Accra temperature data, E_1 was 0.95. But, when EPM=EPMp+EPMc was calibrated with that same temperature data E_1 increased to 0.99 with the EPM residuals plot almost flattened (Fig. 1b) compared to the EPM residuals plot of EPM=EPMp (Fig. 1a).

Although initially the residuals of EMP=EPMp were modeled independently and then added as a separate term to the EPMp to form a more effiicient EPM equation, no simple expression was found to closely model the EPM=EPMp residuals shown in Fig. 1(a). However, when the old EPMp and the resulting EPMc were then calibrated at the same time (as EPM=EPMp+EPMc), the scatter in the EPMp residuals was reduced and a more definite pattern emerged in the seasonal fluctutions which were then more easily modeled with a simple expression for EPMc. Thus calibrating EPM with both the EPMp and EPMc terms resulted in higher precision and E_1 values than calibrating EPMp and EPMc separately.

3.3 Precision and accuracy during calibration

During calibration the match between EPM and TPM was very good for all the sites as reflected in the performance parameters m, r^2 and E_2 being at their best possible values of 1.00. (Table 1). Even though EPM appeared to have performed equally well at all the sites, its performance at the Accra site was best using the E_1 measure which has a greater power of disrmination than r^2 and E_2. However the difference betwen the best ($E_1 = 0.99$) and worst ($E_1 = 0.95$) performances at calibration was only $\Delta E_1 = 0.04$. The desirable value of c=0.00 mm d^{-1} was also achieved at all the sites. Even though the desirable value of MAE=0 was not achieved at any of the sites the worst value was negligible for practical purposes, at 0.03 mm d^{-1}.

3.4 Precision and accuracy under test

When the calibrated EPM, with the values of χ, k, and b obtained from calibration at each of the six weather stations, was used on new data to predict daily $ET_{o,TPM}$ for one year the values of the performance parameters changed little (Table 1 and Fig. 2). The main measure of performance, E_1, either remained virtually the same or declined by a maximum of only 0.01. The values of r^2 and E_2 were still as high as at calibration ($r^2 = E_2 = 1.00$). In general the value of m also changed by only 0.01, except in one case (Abidjan) where it declined by 0.04. The MAE also remained at the same low value in all but two cases where it declined by just 0.01 mm d^{-1}.

At all sites the differences between $ET_{o,EPM}$ and $ET_{o,TPM}$ were so small that although there were as many as 366 points in the linear regression plots of $ET_{o,EPM}$ versus $ET_{o,TPM}$ (Fig. 2) the plotted points were almost indistinguishable from the 1:1 line. While there appears to be large differences between EPM and TPM for ETo values above 5.3 mm d^{-1} in the case of Abidjan, Cote D'Ivoire, it should be pointed out that the maximum difference was only 0.3 mm d^{-1} which occured at $ET_{o,TPM}$ =6.3 mm d^{-1} versus $ET_{o,EPM}$ =6.0 mm d^{-1}, and that E_1 for the year was as high as 0.96. Also, this involved only 8 out of 366 points (i.e., 2%) and thus no general conclusions can be drawn from this observation especially since it was not observed for the five other locations.

3.5 Similarities and differences with other models

EPM is similar, in form, to simple ETo models that use only temperature and solar radiation input data, such as the Hargreaves (Hargreaves *et al.*, 1985), Turc (Turc, 1961) and Thornthwaite methods (Jacobs & Satti, 2001), but it is different from those simple models in that it is not another empirical temperature ETo model based on actual ETo data (Hargreaves & Allen, 2003; Hargreaves & Samani, 1985; Turc, 1961) but rather simply a shortcut method for estimating daily ETo using the Penman-Monteith equation when the only available data are maximum and minimum temperature data. EPM thus takes the place of the Penman-Moneith equation (eqn. 1) itself, and the nine equations–(2), (3), (4), (5), (11), (12), (13), (15), and (17)–that are used to estimate the unavailable weather data. Therefore EPM is simply an empirical form of the Penman-Monteith equation for use when only temperature data are available and all the other data needed to use PM have to be estimated from temperature data.

4. Conclusions

The empirical Penman-Monteith equation (EPM) developed in this study has a much simpler form than the Penman-Monteith equation. Also several fewer intermediate calculation steps are required to estimate daily reference grass evapotranspiration, ETo, by EPM than by the Penman-Monteith equation when only temperature data are available and EPM is applicable at several sites in tropical West Africa. EPM is not another new ETo equation, but rather simply a less tedius way of estimating Penman-Monteith daily ETo when using only temperature data. For practical purposes there is no difference between the ETo estimates made by EPM and TPM. The most difficult part of applying EPM is the initial calibration for a particular location which can be done easily in Microsoft Excel, after which using EPM becomes as simple as using any of the simple temperature-based ETo models. Those who prefer less accurate ETo estimates from locally-uncalibrated versions of simpler ETo models in order to avoid the more involved computations of the FAO-recommended TPM may find the simplicity of the EPM application of TPM attractive for improving their ETo estimates to achieve higher water-use efficiencies.

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Table 1. EPM parameters and performance for six weather stations, computed from 366 daily data for each station. Calibration (C) and test (T) data for the WRI station were respectively, the average 1998-2006 daily data and 2007 daily data. Calibration and test data for all other stations were respectively, the average 1998-2003 and 2004-2008 daily data.

	Weather station name or WMO ID and Call Letters															
	Accra		Abidjan		Mango		Ouagadougou		Lome		Daloa					
	WRI		655780		653520		655030		653870		655600					
	station		(DIAP)		(DXMG)		(DFFD)		(DXXX)		(DIDL)					
Latitude (°)	5.55		5.25		10.36		12.35		6.16		6.86					
Longitude (°)	0.7		-3.93		0.46		-1.51		1.25		-6.46					
Parameter	C	T	C	T	C	T	C	T	C	T	C	T				
m	1.00	1.00	1.00	0.96	1.00	0.99	1.00	1.00	1.00	0.99	1.00	1.01				
c	0.00	0.00	0.00	0.17	0.00	0.05	0.00	0.01	0.00	0.02	0.00	-0.02				
r^2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
E_1	0.99	0.98	0.97	0.96	0.98	0.97	0.95	0.95	0.97	0.97	0.98	0.98				
E_2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
MAE	0.01	0.01	0.01	0.02	0.01	0.02	0.03	0.03	0.01	0.01	0.01	0.01				
χ	2.57		2.78		2.63		2.99		2.84		2.70					
k	14.00		12.57		11.40		8.00		12.35		12.90					
b	0.63		0.63		0.59		0.55		0.63		0.62					

MAE = Mean absolute error (mm d^{-1}); E_1 = modified coefficient of efficiency; E_2 = coefficient of efficiency; χ, k, and b are EPM model parameters.

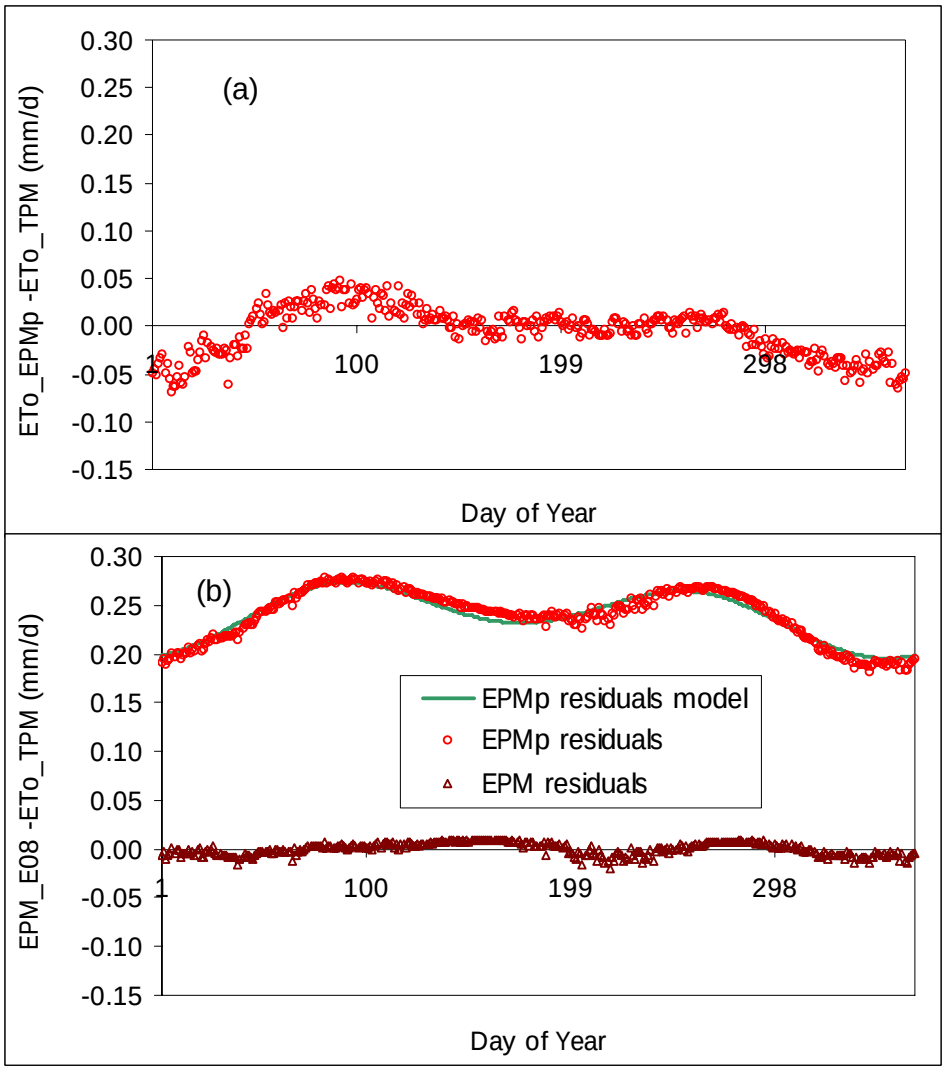

Figure 1. Variations of the daily residuals of the calibrated EPM=EPMp (a) and and of EPM=EPMp+EPMc (b), showing how well EPMc models the modified residuals in the latter case, for the average 1998-2006 data for the WRI weather station.

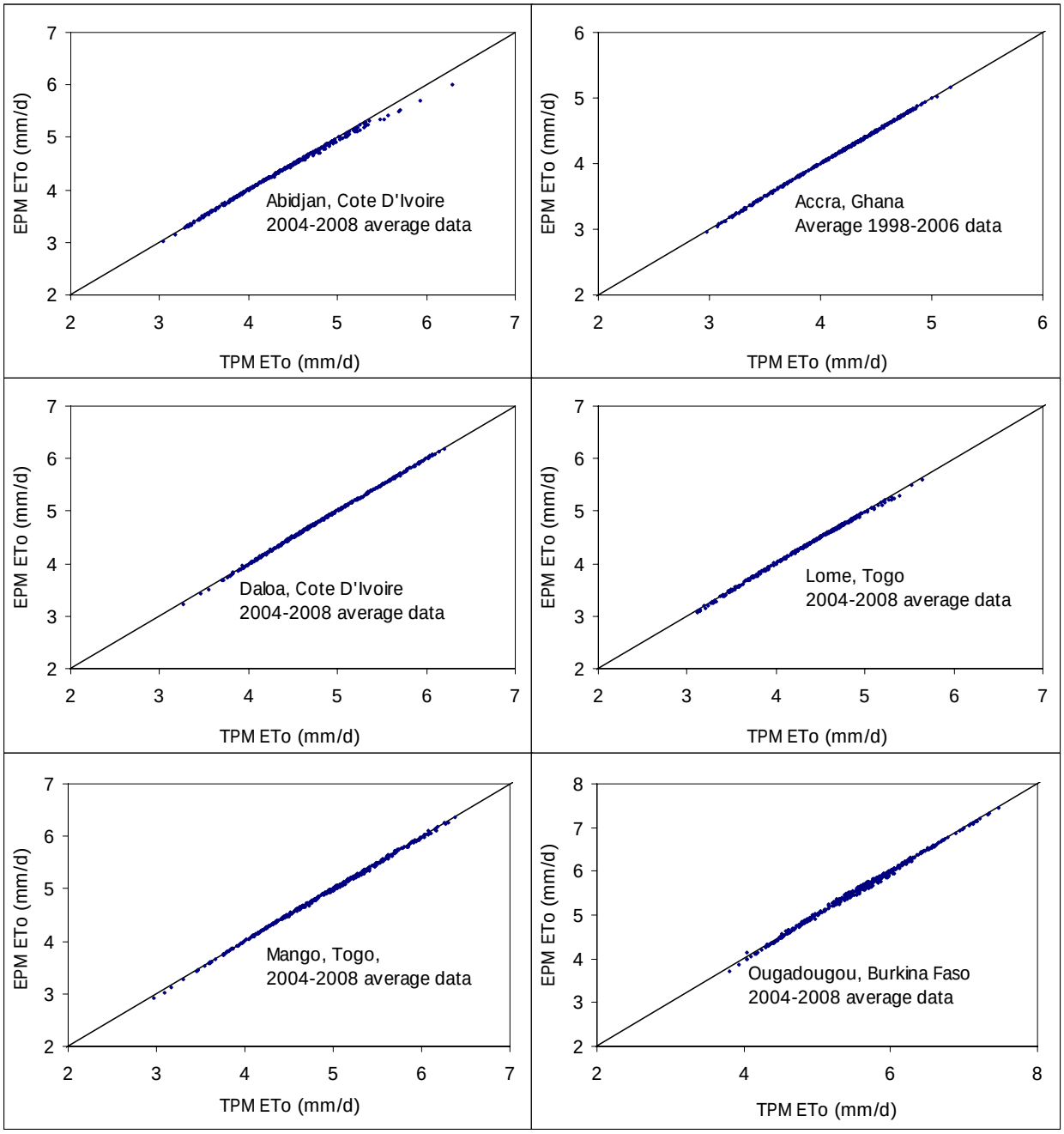

Figure 2. EPM ETo versus TPM ETo for 10-year average daily temperature data for six weather stations in Accra (Ghana), Abidjan (Cote D'Ivoire), Ouagadougou (Burkina Faso), Daloa (Cote D'Ivoire), Lome (Togo), and Mango (Togo). Each plot has 366 data points.

Journal of Agricultural Science
http://www.ccsenet.org/jas
Vol. 2, No. 1
March 2010

The Effects of Temperature Stress on the Quality and Yield of Soya Bean [*(Glycine max L.) Merrill.*]

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Abstract

Reproductive development and growth by crops is especially important for human welfare because we depend on crop fruits and seeds, directly and indirectly, for most of our food. Seed production by crops depends on vegetative development and growth, development of pollen and egg, pollination, and fertilization. The final size of individual seeds generally hinges on cell division within the embryo, followed by seed filling and maturation process. Environmental conditions prior to the shift to reproductive development usually affect by influencing photosynthesis per unit of leaf area, canopy development and interception of solar radiation per unit of ground area, and initiation of potential fruiting site; a strong positive correlation between canopy photosynthesis per unit of ground area and seed number exists for most crops. For many crops where they are now grown, an increase of just a few °C significantly reduce yield. Dependence on soybeans for food and feed has increased rapidly in many countries during the last 30 years. Nutritionists believe that utilization of soybeans should continue to increase in order to provide better nourishment for people throughout the world. For this goal to be realize, present production areas must produce more soybeans and new production areas must be established.

Keywords: Global warming, Pollen, Seed, Quality, Yield, Temperature, Stress, Soybean

1. Introduction

The world today is face with great challenges to produce adequate food, fiber, feed, industrial products, and ecosystem services for the Earth's 6.4 billion people. With nearly 80 million added every year, we must develop ecosystem goods and services to meet the needs of 8 billion by the year 2025 and over 10 billion by 2050 (Reddy, 2005). Climatic changes associated with increasing atmospheric concentration of carbon dioxide (CO_2) and other "greenhouse gases" most importantly global warming and increasing ozone (O_3) concentrations in the lower atmosphere across large crop-growing regions. Many natural and human systems are sensitive and vulnerable to climate change. These changes occurred on local to global spatial scales. Some changes persisted or will persist for only short periods, but others will remain for centuries to come. The magnitude of present population increase is evidence that environmental changes will continue and some change may accelerate in the future because the rates of most processes driving the changes increase with population size. Many environmental changes are innocuous with respect to crop yield, production, and quality, but others are either beneficial or harmful to crops. Observational evidence indicates that recent regional changes in climate, particularly temperature increases, have already affected a diverse set of physical and biological system in many parts of the world (Beggs, 2004). Furthermore, with intensive agriculture, soil degradation and particularly Stalinization are becoming major concerns. Added to these stress comes a threat-global environmental change resulting from increased green house gas concentrations in the atmosphere because of anthropogenic activities.

Global warming will bring about heat stresses on plants that will even affect the type of crops grown. The challenges that global warming poses to agriculture requires that Agricultural Scientists develop response mechanisms to mitigate the changes ion the environment. Hence Plant breeding programmers need to make necessary changes to adopt environment-specific approaches to crop improvement (Reynolds *et al.,* 2001).

The problem of climate change and its possible consequences on agricultural production has received much discussion of late. This problem is real. The obvious result of rising global temperatures will be heat stresses on plants that could alter agriculture with respect to types of crops grown. Agricultural Scientists have to make necessary changes to adopt environment-specific approaches to crop Improvement. Human activities such as deforestation and burning of fossil fuel are mainly responsible for the recent rapid increases in atmospheric concentrations of greenhouse gases including CO_2 (Kaufmann & Stern, 1997; Houghton *et al.,* 2001; Stott *et al.,* 2001). At the present rate of emission, CO_2 concentration is subject to be in the range of 540-970 μmol mol by the end of this century, which will potentially increase global near surface temperature by 1.4-5.8°C (Houghton *et al.,*2001) with some degree of delay, global warming will occur concurrently with increase in CO_2. Therefore, it is important to quantify the interactive effects of increasing temperature and CO_2 on crop production.

In contrast, post-flowering conditions affect yield mainly by influencing ovary or seed abortion, or by changing seed filling duration (SFD) or seed filling rate (SFR). In general, SFD is more plastic than SFR. In spite of the importance of individual seed size, yield variation is more often due to changes in seed number per unit of ground area than to individual seed mass at maturity. Warming hastens crop development and therefore shortens the SFD. Seed number can be reduced via the direct effects of high temperature on reproduction, particularly pollen formation and function. Because warming speeds reproductive development, the seeds that do develop are often small. Although SFR is sometimes stimulated by warming, this effect often does not fully compensate for shortened SFD.

2. Global climate change and crop production

Global climate change has emerged as an important environment challenge due to potential impact on biological systems of planet Earth (Houghton *et al.,* 2001). Since the beginning of the industrial revolution (about 1750), the concentrations of CO_2, methane and nitrous oxide have increased by 31%, 150% and 16%, respectively. The present day CO_2 concentration (370 μmol $mol^{-1)}$ has not been exceeded during the past 420,000 years and likely not during the past 20 million years (Petil *et al.,* 1999). Temperature is the important controlling plant growth and development. Suitability of a crop to a given location depends not only on the threshold temperatures but also on the length of the growing season. Daily or seasonal temperatures above optimum and temperature extremes, should they coincide with critical stages of plant development, will become a major factor limiting crop production.

Several new studies have shown that the climate record of 20^{th} century cannot be explained solely by accounting for solar variability, volcanic eruptions and El Niño cycles. It appears more likely that greenhouse gases from human activities were the dominant drivers of these global-average temperature changes during the 20^{th} century. Future increase in greenhouse gases are projected to raise earth's surface temperature to anywhere between 1.5 to 11º C by 2100 (Stainforth *et al.,* 2005) that would severely reduce soybean crop production. Interactive effects of temperature and [CO_2] on other legume (Ahmed *et al.,* 1993; Prasad *et al.,* 2002, 2003) showed that that the positive interactions observed between [CO_2] and temperature on vegetative growth can not be translated to reproductive process. The physiology effects of high- temperature stress on reproductive development under typical field conditions are more pronounced the effects on vegetative development in many crop species (Hall, 1992).

Most studies on the effect of temperature on soybean seed yield have concentrated on increases in day temperature or concomitant increases in day/night temperature. There were no beneficial interaction between [CO_2], Temperature and UV-B radiation on reproductive development processes such as pollen production, germination and tube length of soybean. Prasad *et al.* (2000) investigated the effect of daytime soil and air temperature of 28 and 38°C, from start of flowering to maturity, and reported 50% reduction in pod yield at high temperatures.

3. Green house effect on crop production

The greenhouse effect is an increase in the average temperature of the earth. It happens because certain gases absorb infrared heat that would normally be radiated into space. Infrared light is what you feel as heat from heat lamps used in restaurants to keep French fries hot. It also causes the heat you feel from ordinary light bulbs. Since carbon dioxide absorbs this heat, the more carbon dioxide there is in atmosphere, the warmer the air will be. If the air gets too hot, the balance of life will be disrupted. Species of plants and animals will die. The food chain could be upset. This would cause many serious problems worldwide.

Between 1961 and 2000, average world crop yields grew rapidly, much more quickly than they had in the preceding millennia that humans have been growing domesticated crops. The rapid growth was a result of the *Green Revolution*, a concerted international effort to exploit advances that had been made in crop breeding, fertilizers and herbicides. The Green Revolution strategy emerged from a surprising confluence of different lines of agricultural research (Evans, 1998) – the development of cheap nitrogenous fertilizers, of dwarf varieties of major cereals, and of effective weed control. Nitrogenous fertilizers increase crop production substantially, but make plants top-heavy, causing them to fall over.

The development of dwarf varieties solves this problem, but at the cost of making plants highly susceptible to weeds, which grow higher than the dwarf plants, depriving them of light. The development of effective herbicides removed this problem. Further Green Revolution development focused on crop breeding to increase the harvest index – the ratio of the mass of grain to total above-ground biomass.

4. Temperature stress on reproductive growth

Plant Reproduction is highly vulnerable to environmental conditions such as temperature and consequently, planet warming may have significant consequences on the reproductive phase with serious implication in agricultural crops. Although pollen tube growth is clearly affected by temperature, little information is available on its effect on the female side and on flower receptivity (Hedhly *et al.* 2003).

Decreased fruit- set at higher temperature was mainly due to poor pollen viability, reduce pollen production and poor pollen tube growth, all of which lead to poor fertilization of flowers (Prasad *et al.,* 2003). Flower abortion also has been attributable to the decreased seeds per plant and seed yield in other crops such as *Brassica napus* (Angadi *et al.,* 2000) *B rapa* (Morrison and Stewart, 2002) and B. Juncea (Gan *et al.,* 2004).

Pollen development, fertilization, and asynchrony of stamen and gynoecium's development are sensitive to temperatures during flowering (Prasad et al., 1999; Croser *et al.,* 2003; Boote *et al.,* 2005). The lost of pollen or stigma viability under high temperatures stress might be the primary reason for the lowered number of seeds produce in the legume (Srinivasan *et al.,* 1998; Davies *et al.,* 1999; Hall, 2004).

Significant negative correlation between pollen production and temperature were found in groundnut (Prasad *et al.,* 1999).Lower seed yield at high temperature under both ambient and elevated [CO_2] conditions was shown to be due to decreased pollen viability in groundnut and bean (Prasad *et al.,* 2002, 2003). Pollen sterility and pollen production at high temperatures may also be associated with early degeneration of the tapetal layer of pollen (Porch and Jahn, 2001).The exact physiological reasons of pollen viability loss are not clearly known and need further investigation (Sailaja *et al.,* 2005).

Reproductive growth leading to seed yield is often depressed by the same increase in temperature that enhances vegetative growth and development. Flower initiation was reduced by temperature > 32°C and seed formation was delayed at 40-30°C (Thomas *et al.,* 2003). When soybean plants were exposed to temperature of 35°C for 10h during the day, yield reductions of about 27% were measured (Gibson and Mullen, 1996). Hence, it is essential to protect crop yield from higher and more frequent episodes of extremely higher temperature both in current and future climates (Salem *et al.,* 2007).

Gan *et al.* (2004) found that the seed yield of canola decrease by 15% when high temperature stress was applied before flowering, whereas the yield reduction was 58% when the stress was delayed to the period of flowering, and further to 77% when the stress was delayed to the pod developmental stage. Physiologically, the high temperature stress during reproductive development may have affected flower abortion, sequent sink site, and later pod abscission resulting a decreased number of seeds per plant (Duthion and Pigeaire, 1991). Also, high temperature stress during reproductive development may have negatively affected cell expansion, cotyledon cell number and thus seed filling rate, resulting in the lowered weight per seed (Munier- Jolain and Ney1998).

Successful fruit set depends on several reproductive processes including pollen germination and tube growth processes. Sexual reproduction in plants is more sensitive to high temperatures than vegetative process, and therefore plant reproductive organs will be more vulnerable to changes in short episodes of high temperatures prior to and during early flower stage (Reddy and Kakani, 2007).

5. Global warming effect on soybean production

Soybean *(Glycine max L.) Merrill,* has become the major source of edible vegetable oils and high protein feed supplements for livestock in the world. About 90% of the world's soybean production occurs in the tropical and semi-arid tropical region, which are characterized by high temperature and low or erratic rainfall. In the tropic, most of the crops are near their maximum temperature tolerance; therefore crop yield may decrease even with minimal increases in temperature.

Temperature has a great influence on the distribution, growth, yield and quality in soybean. It is sensitive to temperature change. The suitable temperature for soybean is 15-22 °C at emergence, 20-25 °C at flowering, and 15-22 °C at maturity (Liu *et al.,* 2008). Soybean seed yield components are also influenced by temperature. Soybean seed yield increased as temperature increased between 18/12 (day/night) and 26/20°C, but yield decrease (when plants were grown at temperature) greater than 26/20°C (Huxley *et al.,* 1976; Sionit *et al.,* 1987). Raising temperature from 29/20 to 34/20°C during seed fill decreased soybean seed yield (Dornobos and Mullen, 1991).

Reproduction plays an important role in the survival and succession of seed crop plants. The onset of the reproductive phase, its duration, and the quality and quantity of reproductive products are regulated by abiotics factors. Of the various abiotic factors, atmospheric temperature and CO_2 concentration are subject to change in the near future. The climate change factors being tested in this study modify reproductive organs and processes. Elevated [CO_2] and high temperature increased flower production in soybean (Nakamoto *et al.,* 2001; Zheng *et al.,* 2002).

Successful cultivation of soybean in the tropic requires the availability of high quality planting seed. Local production of such seed requires cultivars capable of enduring adverse climatic conditions usually present during the later stages desirable in the subtropics because high soil temperature at planting and crusting of the soil surface may result in poor stands (Koti *et al.,* 2004).

6. Temperature stress on seed yield and pollen quality

Seed yield of determinate soybean is produced on both the main stem and branches originating from main stem nodes (Board, 1987). Branch initiation usually occurs first at the cotyledon node prior to vegetative growth stage (Fehr *et al.,* 1977), followed by branch initiation at the unifoliolate node between growth stages (Acock and Acock, 1987). However, most branch vegetative growth does not occur until between reproductive growth stages (Fehr *et al.,* 1977). A majority of the seed yield of determinate soybean is produced on these branches originating from the main stem (Board, 1987). Stresses that reduce crop growth rate between growth stages result in the greatest seed-yield decreases (Linkemer *et al.,* 1998). These results indicate that branch seed yield of determinate soybean is dependent on the amount of branch vegetative growth that occurs during the flowering and pod formation stages of development. Less is known about the effects of temperature stress on soybean branch growth and branch seed yield or how temperature stress affects the distribution of seed yield between the main stem and branches (Frederick *et al.,* 2001).

The number of pods per plant generally increased as temperatures increased to near 26/20°C. Plants grown at temperature exceeding 26/20°C had decreased pod numbers (Huxley *et al.*, 1976; Thomas and Raper, 1978; Sionit *et al.,* 1987). During flowering and pod set, temperatures as high as 30/20°C favored greater pod set (Lawn and Hume, 1985), but temperatures above 40°C severely limited pod formation (Mann and Jaworski, 1970). Seeds per plant increased as temperatures from early vegetative growth to maturity increased from 18/12 to 26/20°C and 26/19 to 36/29°C (Baker *et al.,* 1989). Season- long night temperature increases from 10 to 24°C did not influence seed number (Seddigh and Jolliff, 1984). In contrast to these findings, Huxley *et al.* (1976) observed fewer seeds per plant when day temperature was raised from 27 to 33°C and night temperature was increased from 19 to 24°C. Increase in temperature from 29/20 to 34/20°C during seed fill resulted in fewer seeds per plant (Dornbos and Mullen, 1991). Seeds per pod was the seed yield component least affected by temperature (Huxley *et al.,* 1976; Sionit *et al.,* 1987; Baker *et al.,* 1989).

Weight per seed in soybean was increases in season-long temperatures from 18/12 to 26/20°C (Sionit *et al.,* 1987), but as temperature increased above 26/20°C, weight per seed decreased (Hesketh *et al.,* 1973; Huxley *et al.,* 1976; Baker *et al.,* 1989). Temperatures above 30/25°C during flowering and pod development reduced weight per seed, regardless of the temperature during seed fill (Egli and Wardlaw, 1980). Temperatures above 29/20°C during seed fill decreased soybean weight per seed (Dornbos and Mullen, 1991). Unfavorable growing conditions, such as late planting, excessive soil water, and high plant populations, reduce soybean seed yield primarily by reducing branch growth and branch seed yield per plant (Frederick *et al.,* 1998; Linkemer *et al.*, 1998). Stress that reduce crop growth rate between growth stage R1 and R5 result in the greatest seed- yield decreases (Board and Harville, 1998).

A major obstacle to the expansion of soybean [*Glycine max (L.) Merrill*] production to new areas of the tropic is the difficulty in producing high quality seed. Tropical conditions of high relative humidity and temperature during soybean seed production both before and after the reaches a harvestable moisture level are not conducive to production of high quality seed necessary to establish acceptable stands (Tekrony *et al.,* 1980). Seed vigor is an important parameter which needs to be assessed to supplement germination and viability tests to gain insight into the performance of a seed lot in the field. Seed quality has great impact on the quality of planting stock. The uniformity of seed development within the crop is a major factor through which crop production practices and growing condition will affect seed-to-seed variation. During the growth of field crops, maximum seed quality is generally regarded to be attained at physiological maturity (PM), i.e., at the end of seed filling (Egli, 1998).

The crop is harvest at harvest maturity (HM), when seeds have dried to a moisture content that allows harvesting without considerable damage. By the time seed quality may already have deteriorated. Because seed development within a crop is not uniform, there are differences in the moment individual seeds reach PM. In soybean, longer exposure of early pods to deteriorating conditions was thought to explain the lower viability at harvest of seeds from earlier compared with late pods (Illipronti Jr *et al.,* 2000). Poor seed quality can be cause by the effect of pathogens before physiological maturity, pre-harvest weathering, mechanical damage during and after harvest and deterioration during storage. Hot, dry weather during seed maturation can also result in poor quality (FAO, 1994). It is unlikely that temperature has much effect on seed quality, although that possibility has not been explored. Seed quality is however, sensitive to temperature during the seed-filling period. Because high temperature can differentially affect the various processes involved in seed filling, warming can affect seed composition. In addition seeds are smaller at high temperature, their milling quality can suffer.

In seed plants, successful fertilization requires correct regulation of pollen tube growth. At germination and during growth, the pollen tube interacts with tissue from the pistil while the pollen tube extends via tip growth. Despite the fact that much research has been devoted to the mechanisms regulating pollen tube growth, many aspects are currently unknown.

Plant growth and development, particularly reproductive processes such as pollen grain, pollen tube growth, and fruit set are effected by the temperature more than by any other environmental factor when water is not a limiting factor. Environmental stresses prevailing during pollen development, germination and pollen tube growth affect the functioning of the pollen and eventual fruit and seed set.

Temperature has a clear effect on pollen tube kinetics, expressed as the time required for pollen germination and the rate of pollen tube growth. While temperature affects pollen tube kinetics, information on the effect of temperature on pollen tube dynamics, expressed as the census of the microgametophyte population that succeeded to reach the base of the style, is missing (Hedhly, *et al.,* 2004).

Pollen grains once released from anthers act as independent functional units and are exposed to ambient environment. Therefore, episodes of high temperature during flowering would more severely affect pollen than the deeply seated ovules. Recent studies have shown that micro-and mega-sporogenesis are injured by high temperature, resulting in reduced fruit set (Cross *et al.,* 2003; Young *et al.,* 2004) but they also suggest that pollen plays a major role in fruit-set under high temperature conditions.

Yield decrease due to high temperature and [CO_2] could be due to the effect of on reproduction at both organ and process levels. High temperature inhibits pollen germination and pollen tube growth and genotypes differ in their sensitivity (Huan *et al.,* 2000; Kakani *et al.,* 2002). Plants to exhibit greater reproductive survivability at extreme (low and high) temperatures normally encountered during plant reproduction and for processes leading to yield such as pollen grain development, pollen germination, pollen tube growth, fertilization and embryo development, and finally seed development.

7. Conclusion

Future population growth will place additional demands on crop production. There is scope for both increase yield and expanded cropland area, though that scope varies among regions. In addition to increasing food demand, both population and standard of living increase are bringing with them environmental changes at local, regional, and global spatial scales, and across temporal scales ranging from years to centuries. Many of those environmental changes have important implications, some positive and others negative, for future crop yield and production. Crop breeding and management have the potential to mitigate the negative and reinforce the positive effects of environmental changes on crop physiology and yield. Adaptation by farmers and researchers may be the most important response to these changes. The success of such adaptation might be fostered by basic understanding of effects of environmental changes on crop physiology and growth. This is, at least, one view. I n some case, the degree to which adaptation will be successful, or even possible, may depend on the rate of environmental changes (e.g., the rate of warming during coming decades). Unfortunately, certain knowledge of those rates will be obtained only with the passage of time, and by then it may be too late for effective action.

A better understanding of the influence of high temperature during reproductive growth on soybean seed yield and quality is needed. Although pollen may successfully fertilize an ovule, enhanced high temperature may stay reduce seed production by inducing abortion? The clear effect of high temperature on pollen production and pollen grain germination will have major implications on the fertilization process and fruit set in sensitive crop under future climates. Further studies to relate pollen germination to fruit set are needed. The practices of high-input agriculture are causing concerns about the sustainability of crop production. There are many negative effects on the environment, including pollution by pesticides, emission of greenhouse gases, soil degradation, air pollution by dust, and loss of landraces and other biodiversity. People need to develop new techniques that will keep agriculture both profitable for the farmer and make it sustainable for the future.

This paucity of information along with these variable responses makes it difficult to predict the consequences of enhanced high temperature on pollen production and plant reproductive success. In reality, plants in nature are exposed to multiple environmental conditions concomitantly, and their performance can be assessed only when plants are grown in multiple stress conditions. The impact of global warming will impose great physiological constraints to crop productivity in the tropic. To be successful the researchers will need funding support from government and they will also need to forge linkages with other scientists dealing with this problem in the world. New biotechnological techniques will also need to be applied to face the challenge of global warming. Giving the nature of agricultural production in much of the world today and the challenges facing agriculturally-based economics, we can not be sanguine about the prospects for agricultural productivity, the availability of cropland, or for the environment. Through policy, politic, and global cooperation, we may reduce the environmental problems that cause global warming.

Acknowledgement

The authors are grateful to the Oil Crops Development Project (UTF/MYA/006/Mya) in Myanmar initiated by the Ministry of Agriculture and Irrigation, Myanmar and technical assistance by FAO for granting a fellowship for the first author.

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Journal of Agricultural Science
http://www.ccsenet.org/jas
Vol. 2, No. 1
March 2010

Influence of Seedling Age at Inoculation and Cultivar on the Pathogenicity of a Virus Causing Yellow Mosaic Disease of *Commelina Benghalensis* L. on Cowpea

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Abstract

A screenhouse experiment was conducted to evaluate the influence of seedling age at inoculation and cultivar on pathogenicity of the virus causing yellow mosaic disease of *Commelina benghalensis* L, a broad leaf weed, on cowpea. Three cowpea varieties namely Vita 5, IT84S2246D and Ife Brown were grown in pots and inoculated with sap extracted from leaves of *C. benghalensis* infected with yellow mosaic disease at 7, 14, and 21 days after germination (DAG). It was found that inoculation of cowpea seedlings at 7 DAG subsequently led to the most severe symptoms, which were manifested by mosaic and yellowing of leaves and eventual poor growth and yield attributes. On the other hand, plant growth and yield attributes that were comparable to those of the healthy control plants were recorded for plants inoculated at 21 DAG. Specifically, in regards to the interaction effects, cv. Vita 5 that were sap-inoculated at 7 DAG had the lowest yield attributes, while cv. IT84S2246D inoculated at 21 DAG had the highest yield attributes. The results put together showed that although the yellow mosaic virus of *C. benghalensis* was sap-transmissible and pathogenic to cowpea causing characteristic yellow mosaic disease symptoms and reduction in yield attributes, severity of the disease is less if infection occurs at older stage of cowpea growth.

Keywords: Yellow mosaic virus, *Commelina benghalensis,* Pathogenicity, Seedling age cowpea

1. Introduction

Cowpea, *Vigna unguiculata* (L) Walp (Fabaceae), is an important grain legume in tropical countries of Africa and a veritable source of dietary protein, calcium and iron for the teeming population of human and livestock (Murdock *et al.*, 1997). In Nigeria, the cultivation of this legume extends from the rain forest zone, where its production is marginal, to the Northern Savanna grassland, where over 90% of total seed is produced (Ebong, 1968). The situation remains little changed even now. Practically, most of the cowpea produced is grown in the multiple cropping systems. However, the crop continues to be highly susceptible to a wide variety of pests and diseases both in the field and during storage (Adedire and Ajayi, 2003).

The relationship between crops and disease organisms is generally a complex annual cycle involving crops, vectors and weeds. Weeds are known to play important roles in the spread and epidemiology of virus diseases (Dufus, 1971; Sidek *et al.,* 1993). Many insect and nematode pests, which infect crops with viruses causing major diseases, have often been

shown to acquire the viruses initially from weeds (Zimdahl, 1980). The presence of okra mosaic tymovirus in three malvaceous weeds in Nigeria appeared to be an important source of the virus for crop plants (Atiri, 1984). Cowpea chlorotic mottle bromovirus (CCMV), which causes severe damage in susceptible cowpea cultivars alone or in mixed infections, has been isolated from two weed species, *Clitoria ternatea* and *Desmodium heterocarpon* also in Nigeria (Thottappilly *et al.,* 1993). In the United States of America, cucumber mosaic virus, which causes epidemics of disease in Cantaloupe melons, lettuce and sugar beet, is carried by at least two aphid vectors from several weeds including species of *Brassica*, *Sisymbrium* and *Physalis*. Certain garden ornamentals and several crops also carry the virus in Arizona (Rice, 1974).

Commelina benghalensis L., Family- Commelinaceae, an annual /perennial weed, occurs widely in the tropics where it grows in a wide range of situations in grassland arable crops including cowpea fields. *C. communis,* a close relative of *C. benghalensis* has been reported as a naturally infected weed host of CMV (Zitter, 2001), which is also an important pathogen of cowpea both experimentally and in the field. The *Commelina* yellow mottle virus is a DNA virus and it has been isolated from this weed. Apart from the allelopathic activity of this weed on crops it appeared to be an important virus reservoir, thereby constituting a major constraint to efforts aimed at increased and sustainable food production.

The age of plant at inoculation, genotype, season of growth, planting density among other factors have also been found to exert varying degree of influence on the pathogenicity of disease agents in different pathosystems. Many specific relationships between weeds, crops and their pests and pathogens have been established, sometimes enabling action to be taken against weeds to prevent or reduce damage to crops (Mattews, 1991). The objective of this study, therefore, was to determine if the pathogenicity of the mosaic disease of *C. benghalensis* on cowpea was influenced not only by the host cultivar but also by the age of seedling at inoculation as well as their possible interactive effects.

2. Materials and Methods

2.1 Experimental design and plant propagation

Experiments were conducted in the screenhouse of the Faculty of Agriculture, University of Ilorin, Nigeria situated in the Southern Guinea Savannah ecological zone, to evaluate the influence of age of seedling at inoculation on the pathogenicity of the causal agent of a yellow mosaic disease of *C. benghalensis* (weed host) in 3 cultivars of cowpea. The cowpea cultivars were inoculated at 7, 14, and 21 days after germination (DAG). It was factorial a 3x4 factorial experiment in a completely randomized design with 6 replications. The three cultivars of cowpea were Vita 5, IT84S2246-D and Ife Brown, which were obtained from the Teaching and Research Farm of the University of Ilorin, Nigeria. They were raised in stands of two in 5-liter (25 cm diameter) plastic pots, filled with sandy-loam soil sterilized at 121^{o}C for 30 minutes prior potting.

2.2 Collection of diseased leaf samples and inoculation

Diseased leaf samples of *C. benghalensis* naturally manifesting yellow mosaic disease symptom were collected from farms adjoining the Faculty of Agriculture buildings, University of Ilorin. Leaf sap was extracted by homogenization of leaves using mortar and pestle, in 0.05 M phosphate buffer, pH 7.2, at the rate of 1 g leaf sample to 1 ml of the buffer.

The first and second leaves from the stem base of cowpea plants were lightly dusted with carborundum and rubbed with the extracted sap using cotton wool at the 2-3 leaf stage (7 DAG), 4-5 leaf stage (14 DAG) or 6-7 leaf stage (21 DAG). The leaves were rinsed with running water. The control plants were mock –inoculated with phosphate buffer only. Plants were watered once per 3 days to avoid water stress during the growth period of the plants.

2.3 Data collection and analysis

Initially, observations were made daily and then on weekly basis for symptoms manifestation i.e. type and nature of symptoms, number of days to appearance of symptoms after inoculation and position on plant of the leaf with first symptoms. Growth parameters such as plant heights, number of leaves and mean leaf size using leaf area meter, were taken on a weekly basis after each inoculation and at flowering and podding stages. Yield parameters including the number of pods per plant, total pod weight per plant, average weight per plant, percentage grain weight per pod, and percentage yield loss per variety and inoculation regime were determined at or after harvest. All collected data were subjected to analysis of variance (ANOVA). Treatment means were separated using the New Duncan's multiple range test at 5% level of significance.

3. Results and Discussion

3.1 Symptoms manifestation

Symptoms of the disease caused by virus extracted from weed host, *C. benghalensis* appeared on sap- inoculated cowpea plants. Mock- inoculated plants were however free from the visible symptoms of the disease. Initially, the symptoms manifested as mild mottling graduating to pronounced yellow mosaic pattern with increased age of infection (Plate 1). These were similar to the symptoms observed on naturally infected weed host (Plate 2).

First symptoms appeared on cowpea plants inoculated at 7 DAG at 6.3 days after inoculation while it appeared at 9.0 and 11.0 days after inoculation respectively on cowpea plants inoculated at 14 and 21 DAG (Table 1). The three cowpea cultivars varied significantly in respect of days to appearance of symptoms on plants after inoculation. The disease symptoms appeared at 6.6, 9.0 and 11.0 days after inoculation on cultivars Vita 5, IT84S2246D and Ife Brown, respectively (Table 1). The interaction effect between cowpea cultivar and seedling age at inoculation was significant. On all cowpea cultivars, symptoms appeared earliest in seedlings inoculated at 7 days after germination, followed by those inoculated at 14 days and 21 days after germination. Cultivar Vita 5 inoculated at 7 dag was the first to manifest symptom in 5 days after inoculation, while Ife brown inoculated at 21 DAG was the last in13.8 days after inoculation.

3.2 Plant growth response

The combined effects of age of plant at inoculation and variety on plant height, number of leaves and leaf size of 3 cowpea cultivars over the first 5 weeks following inoculation is shown in Table 2. The values for cv. Vita 5 inoculated at 7 DAG were the lowest whereas cv. IT84S2246-D inoculated both at 14 and 21 DAG were the highest among inoculated plants. In all cases, the mock-inoculated plants of all varieties had the highest values followed by those inoculated at 3 weeks after germination. Plate 3 compares the appearance (plant height, number of leaves, size of leaves etc) of infected and healthy control cv. Vita 5 plants at 5 weeks after inoculation with the viral agent.

3.3 Yield response

The main effect of age and variety as well as their interaction effect on yield responses of cowpea are shown in Table 3. For all recorded parameters, which included total number of pods per plant, total and average weight of pods, total and average grain weight as well as % weight of grain per pod, the general trend was similar to that recorded for growth parameters. Significantly lower values were recorded for plants that were inoculated at the earliest seedling age compared to those inoculated later. Analysis of the varietal effect showed cv. vita 5 with significantly lower yield values than the other two cultivars for most of the parameters while Cv IT84S2246D had the highest values. Specifically, cv. Vita 5, sap-inoculated at 7 DAG, had the lowest yield value while cv. IT84S2246D inoculated at 21 DAG had the highest yield values for most parameters.

3.4 Comparative percentage yield loss

The percentage loss of yield based on grain weight, pod weight and number of pods, in the three cultivars of cowpea is shown in Fig. 1. The three cowpea cultivars significantly differed with regard to each parameter. In all cases, cultivar IT84S2246-D had the lowest percentage of losses, while the highest percentage loss was recorded in cultivar Vita 5.

It is apparent in this study that the degree of susceptibility varied with the cultivars in terms of time of appearance of symptoms, and the subsequent growth and yield responses. Inter cultivar comparison showed that cv. Vita 5 was the most susceptible at all inoculation stages. Relative to the control in each cultivar, grain yield loss due to early infection was in the range of 40 to 60 %, while late infections caused between 10-15 %. Cowpea plants inoculated early (7 days after germination) with the sap from infected weed, manifested early mosaic symptoms, while plants inoculated late (21days after germination) manifested symptoms much later. Such plants that manifested early infection symptoms subsequently had lower growth and yield components than those recorded for plants that had late symptom manifestation, owing to inoculation at relatively older age. This observation has further highlighted the role of physiological maturity conferred on the host plant system by age. A similar observation to that observed here was made long ago (Owusu *et al.*, 1968), who reported that the earlier tobacco plant was infected with the tobacco ring spot virus, the higher the pathogenic effect of the virus on the tobacco plant. Of recent, similar observations were also made on some tomato cultivars mixed infected with the tomato mosaic virus and potato virus X (Balogun, 2008).

A reduction in total fruit yield is a common feature, and an important economic aspect of virus diseases (Mathews, 1991). Yield reduction manifesting in various forms is therefore normally expected with increased disease severity in virus diseases, as was the case in this study, in which the severity was enhanced by infection at early growth stage. Reduction in size and number of pods was most noticeable and this was probably a result of impairment of pod and seed initiation, as well as increased abortion as had been observed by Walkey *et al.* (1985). Hampton (1975) had noted that lower yields in virus infected cowpea may sometimes be due to a reduction in both the size and number of fruits.

The results in the present study support earlier observations and reports of Balogun and Aliyu (2005) that the broad-leaf weed, *C. benghalensis,* naturally infected with the yellow mosaic disease could serve as a potent reservoir for the causal agent and a source of inoculum for transmission to susceptible arable crops. More over, the fact that the viral agent was easily transmitted by rubbing the juice from the infected weed, on cowpea leaf, under ambient screenhouse conditions makes it highly plausible that transmission through farm operations, such as manual weeding, which is still widely practiced under the vastly subsistent farming system in sub-Saharan Africa, was a possibility.

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Table 1. Days to appearance of first symptoms in 3 cowpea cultivars after inoculation with sap extract from C. *benghalensis* naturally infected with a yellow mosaic disease

- = Did not manifest symptoms.

Cultivar / Days after germination	Days after inoculation to appearance of first symptoms[1]			
	Vita 5	**IT84S2246D**	**Ife Brown**	**Mean[2]**
Mock inoculation	-	-	-	-
Inoculation at 7 DAG	5.0g	7.6de	6.4f	6.3C
Inoculation at 14 DAG	7.0ef	11.5b	8.8c	9.0B
Inoculation at 21DAG	7.8d	13.8a	11.5b	11.0A
Mean[3]	6.6γ	11.0α	8.8β	

[1]Figures within columns and rows having the same small letter(s) do not differ significantly (P>0.05).

[2]Means within the column having different capital letter differ significantly (P<0.05).

[3]Means within the row having different Greek letter are significantly different at P< 0.05) using the New Duncan's Multiple Range test.

Table 2. Combined effect of age of plant at inoculation and cowpea cultivar on some growth parameters at 5 weeks after final inoculation

Cultivar x Seedling age at inoculation	**Plant height (cm)**	**No. of leaves per plant**	**Average leaf size (cm^2)***
Cv. Vita 5, Mock- inoculated at 7 DAG	30.0a	17.5a	52.6a
Cv. Vita 5, Inoculated at 7 DAG	14.4f	13.8d	21.2d
Cv. Vita 5 Inoculated at 14 DAG	14.9f	14.0d	25.4d
Cv. Vita 5 Inoculated at 21 DAG	24.3b	17.0ab	31.0c
Cv. IT84S2246D Mock- inoculated at 7 DAG	31.9a	16.3abc	54.2a
Cv. IT84S2246D Inoculated at 7 DAG	20.9cd	15.8bc	37.1b
Cv. IT84S2246D Inoculated at 14 DAG	22.8bc	14.0d	38.1b
Cv. IT84S2246D Inoculated at 21 DAG	24.6b	16.0bc	48.0a
Cv. Ife Brown Mock-Inoculated at 7 DAG	31.2a	16.5abc	53.4a
Cv. Ife Brown Inoculated at 7 DAG	17.9e	15.5c	24.5c
Cv. Ife Brown Inoculated at 14 DAG	19.5de	13.8d	33.8b
Cv. Ife Brown Inoculated at 21 DAG	23.8bc	15.8bc	37.1b

[1]Means within a column followed by the same letter(s) are not significantly different using the New Duncan's Multiple Range Test at P= 0.05.

* Size of a trifoliate leaf measured by a leaf area meter

Table 3. Effect of age and variety and their combination effects on some yield parameters in cowpea under mock or sap inoculation with yellow mosaic virus disease of *Commelina benghalensis*

Seedling age / inoculation	No of Pods Per Plt	Total Pod Wt(g)	Mean Pod Wt(g)	Total Grain Wt/Plt	% Grain Wt/Pod
Mock Inoculated at 7 DAG	13.5a	15.3a	1.15a	13.5a	88.1a
Inoculated at 7 DAG	7.8d	7.9d	0.97b	6.3d	76.7c
Inoculated at 14 DAG	9.4c	9.4c	0.98b	7.9c	82.6b
Inoculated at 21 DAG	10.7b	10.8b	1.0b	9.2b	82.9b
S.E	0.24	0.25	0.03	0.38	1.31
Cowpea Variety					
Cv. Vita 5	6.6c	6.3c	0.9c	5.1c	76.7b
Cv. IT84S2246-D	13.4a	13.9a	1.0b	12.1a	86.9a
Cv. Ife brown	11.0b	12.4b	1.1a	10.5b	84.1a
S.E	0.2	0.3	0.03	0.3	1.1
Treatment Combinations					
Cv.Vita5 mock-inoculated	9.8de	11.5c	1.2ab	10.3c	89.5a
Cv.Vita 5 inoculated 7 DAG.	4.5g	3.4f	0.74e	2.2e	66.4d
Cv.Vita 5 inoculated 14 DAG	5.5fg	5.0ef	0.84de	3.5e	76.4c
Cv.Vita 5 inoculated 21 DAG.	6.5f	5.6e	0.87de	4.2e	74.5c
Cv.IT84S2246-D mock- inoc.	16.8a	17.1a	1.0bc	15.4a	89.7a
Cv.IT84S2246-D inoc 7 DAG.	10.0d	11.1c	1.1abc	9.4c	84.6ab
Cv.IT84S2246-D inoc 14 DAG	12.5c	12.4c	0.99cd	10.8c	87.1a
Cv.IT84S2246-D inoc 21 DAG.	14.5b	14.9b	1.0bc	12.9b	86.3ab
Cv. Ife brown mock-inoculated	14.0b	17.4a	1.3a	14.8a	85.0ab
Cv. Ife brown inocd 7 DAG.	8.8e	9.1d	1.0bc	7.2d	79.2bc
Cv. Ife brown inocd 14 DAG.	10.3d	11.2c	1.1abc	9.5c	84.4ab
Cv. Ife brown inocd 21 DAG	11.0d	12.0c	1.1abc	10.4c	87.8a

*Means in the same column followed by the same letter(s) are not significantly different at P= 0.05 using the New Duncan's Multiple Range Test.

DAG: Days after germination.

Plate 1. A: Mock-inoculated (control) Cowpea cv. Vita 5 remained without symptoms even at the fruiting stage.
B: Cowpea cv Vita 5, inoculated at 7 days after germination with sap from infected leaf of *Commelina benghalensis*, manifesting severe yellow mosaic symptoms a few weeks after inoculation.

Plate 2. A: Wild *Commelina benghalensis* without virus (mosaic) symptoms (apparently healthy)
B: Naturally infected *Commelina* plant manifesting yellow mosaic symptoms

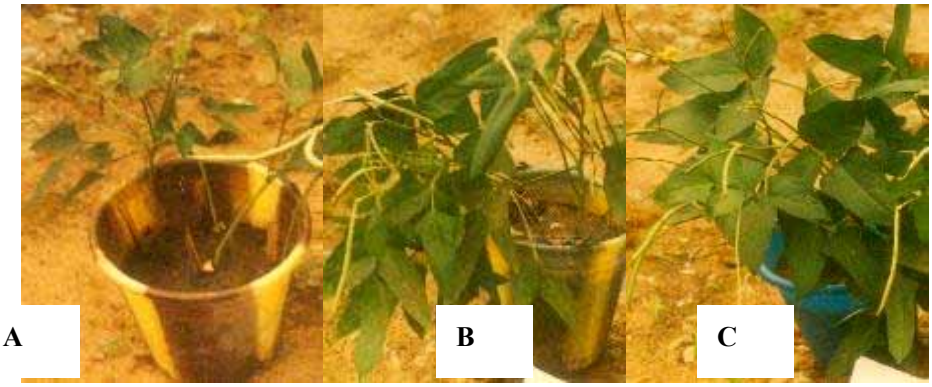

Plate 3. Differential growth and yield response of cv Vita 5 cowpea plants following mock or sap inoculation at different seedling age with buffer or sap extracts from *Commelina benghalensis* weed plants manifesting yellow mosaic disease syndrome.
Seedlings were sap- inoculated at (A) 7 days after germination (DAG) (B) 21 DAG. (C) Mock-inoculated with buffer only at 7 DAG (healthy control). Plants were photographed at the reproductive stage (8 weeks after germination i.e. 5 weeks after the last inoculation).

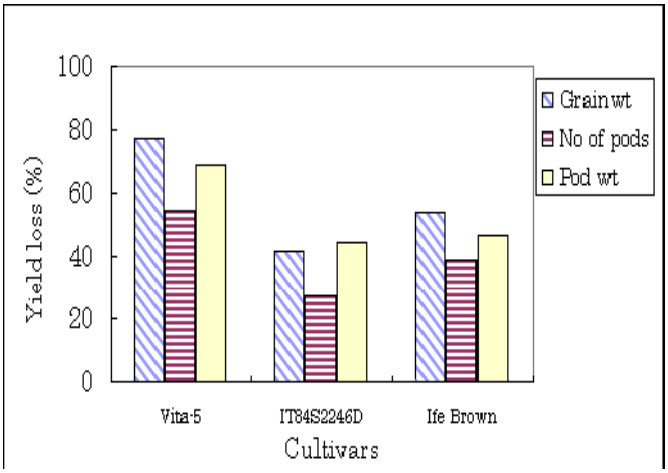

Figure 1. Comparative yield loss in three cultivars of Cowpea under infection with a virus isolate from *Commelina benghalensis,* a weed host

Journal of Agricultural Science
http://www.ccsenet.org/jas
Vol. 2, No. 1
March 2010

Damage and Management of Alien Species in China

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This study was supported by Agricultural Biological Resources Protection and Utilization Project(No. 2130108) and the Open Fund of Key Laboratory of Genetic Breeding and Aquaculture Biology of Freshwater Fishes, Ministry of Agriculture(No. BZ2009-10).

Abstract

Alien invasive species have been a seriously environment issue in the whole world. The ecological damages and economic influences of alien invasive species are analyzed in this article, and the government is suggested to strengthen the quarantine services, strictly manage the intentional introduction of alien species, and implement the risk supervision system for the introduction of alien species.

Keywords: Alien species, Damage, Management

1. Definition and influence of alien species

Alien species mean the species which originally don't exist in the region or the ecological system, but are introduced because of human activities or relative causes about human activities. The alien species from international countries are called as the foreign alien species, and the alien species from the different regions in same one country are called as the regional alien species, and the corresponding specials are called as the native species (Li, 2002, P.211). When alien specials enter into a new region, survive and propagate, and form a feral population which further diffuses, and has formed or will form ill ecological or economic effects, and this event is called as the biological invasion, and the alien species induced the biological invasion are called as the alien invasive species. As viewed from human history, the probability that alien species invaded the local ecological environment was very small, and "the probability that one species becomes the invasive species is only about 1‰ (Lu, 2003, P.35-37)". But with the increasing frequency of international economic communication and the convenience of the traffic, the gradual elimination of original geographic factor, the communication and penetration of species among different regions in the world become more and more frequent, and the invasive risk of alien species increases at the same time (Naylor RL, 2001, P.1655-1656). Alien species usually invade local ecological environment by two approaches, i.e. the artificial factor and the natural factor, and the artificial factor is divided into the unintentional introduction and the intentional introduction, and the intentional introduction has been the main approach that alien species invade China at present. According to the incomplete statistics, the alien invasive ruderals to China are 108 sorts, and 62 sorts of them are intentionally introduced, 58% of the total amount (Cai, 2003, P.27-34). The alien invasive species will induce very serious damages to the human being and the society, which are mainly embodied in the ecology and the economy.

1.1 Ecological influences

1.1.1 The invasion of alien species will induce the loss of the biological diversity in the ecological system

In each one special ecological system, because of the long-term evolution of the nature, biology restrains their natural enemies each other, and is influenced by other factors such as climate, and each population is limited in the special region and quantity. When one species enters into one new region, it will easily become the dominant species because of various causes (such as lacking in sufficient biological resistance) to reduce the species diversity, and make other survived species depending on the local species diversity to lose the comfortable habitat. For example, Christian thought that the reciprocity relation between the seed spreader and the plants in the "animal-plant-seed spread" system was very important to maintain the survival of the species, update the community structure and the diversity in his "Hypothesis of Key Mutual Benefits". The loss of certain one species induced by the invasion of alien species will induce the chained extinguishing disaster of relative species (Christian, 2001, P.635-639).

1.1.2 The invasion of alien species will induce the loss of the genetic diversity in the ecological system and serious biologic genetic pollution

Invasive species will contest with indigenous species for survival space and foods, occupy the ecological niche of local species, and threaten the survival of indigenous species. Invasive species will also directly kill local species (such as eating the ovum, juveniles even adults of local species) to influence the survival of local species, or directly threaten local species, or excrete and release chemical substances to reduce the sorts and quantity of local species and restrain the growth of other species, even make them to be in severely ill or killed out, which will induce the loss of the genetic diversity of the ecological system. Invasive species will also hybridize with indigenous species with close relative, which may induce the genetic erosion of the later, and some genes which can not suit for local environment may be inherited to indigenous species, and the inheritance quality of indigenous species may be reduced, and the genetic pollution will occur, and the genetic diversity of the ecological system will be impacted, so the biological diversity of the ecological system will be reduced.

1.1.3 The invasion of alien species will induce the decrease and loss of the protection ability of the ecological system

Because of the invasion of alien species, many alien invasive species began to destroy the local vegetation, and form the mono-dominant community, and indirectly reduce the sorts and quantity of other local species depending on the local vegetation, and finally the singularity and degeneration of the ecological system will form, and the ability of the biology which resist exterior risks depending on the species diversity in the complex ecological system and the biologic chain will gradually reduce. Invasive species may also bring causal organisms and parasites which will destructively attack indigenous species, and largely damage the local ecological environment, and they impact the species structure and the food web structure of the local ecological system by preying and competition, so the capacity to withstand risks of the ecological system will be reduced.

1.2 Economic influences

Alien invasive species are integrated pests which will directly harm the development of forest economy, and the quick diffusion of alien invasive species may quickly change the natural sight of the ecological system, such as covering rivers and lakes, destroying forests and grasslands, and harming environment and human safety, making the original tourism region to lose value and bringing loss to the tourism industry. Alien species could produce indirect economic loss by a series of bad influences on water and soil and climate changing the ecological system. According to the first alien invasive species survey report in China, there were total 283 sorts of alien invasive species which would produce 119.876 billion Yuan of direct or indirect economic loss, 1.36% of GDP (Xu, 2004, P.138-147 & 166-181), and only several sorts of main alien invasive species would induce 57.4 billion Yuan of economic loss in each year (Li, 2002, 1-9).

At the same time, the ecological effects and economic influences of same one species may be different even completely different because of the difference of time and place. Most introduced alien species are beneficial for human beings, and one species, one variety even one gene may be the headspring to flourish the economy of the country (Xue, 2005, P.585), such as the Tilapia and penaeus vannamei boone. When alien species enter into the new environment, they should possess certain internal and external reasons to become new dominant species. According to Williamson's theory of "tens digit law" (Williamson, 1996, P.1664-1666), once alien species invade the local environment successfully, it is very difficult to fully eliminate their influences, and the costs controlling their damages and diffusions are very large by using large numbers of financial, material and human resources. Therefore, the governments should adopt various effective measures to prevent the possibility of the invasion of alien species.

2. Countermeasures to prevent the invasion of alien species

Aiming at many deficiencies and shortages existing in the legislation about the present alien species invasion of China, the innovation of legal system is the base and key to establish the legal prevention system of alien species invasion. Various parts of the invasion of alien species such as establishing relative legal management measures aiming at each one invasion approach and ensuring the coherence of relative legal articles with corresponding international convention and agreements should be fully considered to completely realize the management of alien invasive species by law.

2.1 Prevention of the "unintentional introduced" species

According to the research and survey report of the key project aiming at the invasion of alien species, 76.3% alien invasive animals entered China with the trading goods or the conveyance because of loose checking (Ou, 2006, P.1240-1244). And the international commercial trading and the travel of tourist are always the convenient approach for the invasion of alien species (Qin, 2004, P.44-47), for example, the golden-rod of Canada was unintentionally carried by tourists and entered China (Li, 2003, P.7-9). Once the checking is loose, such convenient ports and land traffic may intentionally or unintentionally introduce more alien species, and a few of them may induce large damages. Therefore, the import quarantine supervision should be strengthened to prevent the introduction of alien harmful species and reduce the quantity of introduced harmful species to the lowest (Chen, 2005, P.49-50).

Following countermeasures and advices are suggested, (1) establishing the alien invasive species identification and alarming information center, and building the database and information system of the harmful invasive species to college, dispose and feed back relative information and results, (2) strengthening the construction of the ability of alien species risk evaluation, and analyzing the risks of potential invasive species, and perfect the quarantine and the environment supervision measures of alien invasive species, (3) strengthening the relative scientific researches to preventing the invasion of alien species, and closely associating with the institutions which want to intentionally introduce alien species, and cultivating the public's consciousness and conscious behaviors to face the invasion of alien species by various medias and channels.

2.2 Prevention and management of the invasion of "intentional introduced" species

Relative systems should include the ecological safety risk evaluation system before the introduction of species, the emergence reaction system of harmful invasive species, the ecological safety supervision system of introduced species and the elimination system of invaded species, and the prevention, evaluation and supervision of the species invasion.

2.2.1 Establishing the catalog system of species and the risk supervision system

The article 14 of the "National Ecological Environment Protection Program" specially regulated that "the introduction of alien species must be assessed by risk", because the damage of the alien invasive species is very large, and once the damage forms, the possibility of elimination is very small, so it is very important to study which species will be the invasive species, where the invasion will induce damages and what damage will be induced (Xu, 2003, P.4-8). The main points and difficult points of the alien species risk evaluation are the establishment and design of the index system (Ding, 2006, P.92-96), and according to the varieties, introduced approaches, the biological and ecological characters, and the difference of damage characters of alien species, different ecological safety risk evaluation systems (respectively aiming at terrestrial pants, land invertebrate species, aquatic invertebrates, microorganisms, aquatic plats, and aquatic animals) and different licensing systems and access systems with different classes should be established. Because of large differences of the damage states in different ecological systems of alien species, the alien species invasive risk evaluation index system for special region should be established and different species should have different evaluation indexes. The present Weed Risk Assessment System of Australian (Chen, 2001, P.466-471) is composed according to a series of question arsenal including the ecological characters, geographic characters, and biological characters of the species. Agreement on the Alien Pants Evaluation of Hawaii established by Daehler et al (Daehler C C, 2004, P.360-368) is mainly to evaluate the risks of the possible invasive alien plants to Hawaii and other Pacific islands from four aspects including "the influence of invasive species on the ecological system, the living character of species, the potential diffusion ability and the control difficulty". Jiang Qing et al (Jiang, 1994, P.331-334) put forward the harmful species fatalness evaluation index system, Ji Liang et al (Ji, 2004, P.100-105) analyzed the fatalness of quarantine harmful biology, and Xiang Yanci et al (Xiang, 2002, P.40-48) made the ecological risk evaluation and management of alien plants, and the PRA Project Group of "the Eighth Five-Year Plan" of China Ministry of Agriculture had confirmed the harmful biology fatalness evaluation system in 1997. Yu Yinchang et al (Hu, 2006, P.113-115) put forward the alien aquatic animal invasion risk evaluation system according to the fertility, the competitive strength and the viability of aquatic animals.

In conclusion, the invasion of alien species is a complex chain process, and it is a long-term process from colonization, constructive species to diffusion, and many invasive species have a time-lagging term after introduced, and different species have different time-lagging terms such as a few years even tens of years. The damage of alien species to the genetic diversity is irreversible (Liu, 2004, P.94-98). Therefore, the environmental risks of alien species should be evaluated, and only those species with few or small influences on the ecological environment after the environment risk evaluation can be introduced.

2.2.2 Establishing the risk prevention system

First, check the actuality of alien species clear. The detailed information of the alien species in China, such as the invasion varieties, the source areas, the distribution areas and the transmission routes, should be made clear to study the control measures and establish the supervision system aiming the existing invasive species or potential invasive species. Based on that, the prevention and comprehensive treatment of known alien harmful species should be strengthened. Second, confirm the key prevent area of main alien invasive species, and relative departments should confirm and publicize above information. Local governments should strengthen the environment management in the natural reserves, the landscape and famous sceneries, and forest parks to prevent the intentional or unintentional introduction of alien invasive species. Third, strengthen the scientific research and enhance the level of environment management. For the prevention and treatment of alien invasive species, the technology is the first measure. At present, many countries in the world all very pay attention to the communication in this technical domain, and China should establish the integrated management system including ecological system, habitat restoration, biological prevention, low-pollution prevent, ecological replacement, early earning and remote sensing by exterior species environment influence evaluation and foreign alien species control technology.

2.2.3 Establishing the species introduction supervision system

Local governments should establish the stable breeding and representative area system, i.e. in the planned land, adopting the stable breeding measure to the introduced species with uncertain harm in 1~2 years, and supervising their growth and propagation, and creating profiles for these species, especially for those species with strong suitability and fecundity. At the same time, set up the buffer zones surrounding of the breeding area, which can help to control the invasion of alien species. The spread of introduced species must be demonstrated by relative experts and evaluated by relative institutions. The good quick response system aiming at the invasion of alien species should be established, and once alien species have invaded the region or have the threat to invade the region, the relative department should adopt proper measures such as eliminating, restraining and controlling as soon as quickly to reduce the negative influences. For the confirmed alien invasive species, or the alien invasive species which have been controlled, or the alien introduced species with higher hazard index, relative department should strictly supervise them to prevent their spread or repeated invasion. For the alien invasive species (such as Pomacea canaliculata and Cambarus proclarkii) which have induced harms, the comprehensive technology measure should be studied and developed to reduce or eliminate their damages, and for the alien invasive species (such as Brazilian Slider and ashman) with potential harms, relative department should strictly restrain them in the breeding range, and adopt necessary measures to prevent escaping and limit the breeding scale, and implement strict track supervision to eliminate the dangerous alien economic animals in the initial diffusion stage.

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Journal of Agricultural Science
http://www.ccsenet.org/jas
Vol. 2, No. 1
March 2010

Effects of Medicinal Plant (*Kigelia Africana*) on Sperm Quality of African Catfish *Clarias Gariepinus* (Burchell, 1822) Broodstock

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Abstract

The effects of *K. africana* fruit (Lam. Benth), family bignoniaceae, were investigated on the sperm quality of *C. gariepinus*, (mean body weight, 396.05±7.04). Five diets with crude protein of 40% were formulated with different inclusion levels of *K. africana* powder. D1 (control) has 0 g/kg of the powder, while D2, D3, D4 and D5 has 50, 100, 150 and 200 g/kg of the *K. africana* powder. A total of 120 *C. gariepinus* were randomly distributed in triplicate into 15 concrete tanks (2×2×1.5m) at stocking density of 8 fish per tank and constant water level of 1m was maintained in the experimental tanks. The tank contained pond water (PH ≈ 7.2, oxygen≈ 4.3 mg/l, temperature≈ 26. 2 ^{0}C). The fish were fed at 3% of body weight twice a day between 8.00-9.00 am and 4.00-5.00 pm for a period of 90 days. The qualities of the milt were assessed by aid of microscope and by fertility tests. The male brood fish fed 100 g/kg had significantly higher ($P<0.05$) sperm counts (6.5± 1.2x10^{9}sperm/m), % motility (92%), fertilization ability (90.88±1.03), lower milt volume (1.45±0.71) and motility duration of (39.00±1.4). However, significant differences were not observed in the length and weight of the testes among the diet groups ($p>0.05$). The results of the study has shown that *K. africana* fruits possess promising pro-fertility which can be exploited in fish seeds production and 100 g/kg of *K. africana* based diet was the best tolerance level of inclusion, which could give satisfactory and efficient result on the sperm quality and fertility of *C. gariepinus*

Keywords: Kigelia africana, Sperm quality, Fertility, Clarias gariepinus

1. Introduction

Aquaculture is a fast growing sector in Nigeria contributing less than 5% of the total fish supply but at a growth rate of about 2% per year (Moses, 2006). Among the culturable fish in Nigeria includes *C. gariepinus,* which is a major tropical aquaculture species in Africa (Ayinla and Akande 1988) and most popular with fish farmer and consumers.

C. gariepinus commands a very good commercial value in Nigerian markets (Ayinla *et al,* 1994). It has been noted that farming is hardly imaginable without availability of fish seed (Chondar 1980). Based on a 1992 United Nations Development Project (UNDP) assisted base line study, the total annual fingerlings requirement for Nigeria was 250,000 million while the domestic production stood at 7.2 million (Nwokoye *et al.* 2007).

In fish reproduction under controlled conditions, attempts are made to obtain sperm of the highest quality and hence to produce the highest possible numbers of good quality seeds. Several factors that affect fish seeds quality includes

different strains, genetics, nutrition, content of feed and activities of modern agriculture which have introduced several substances such as organic matter, chemical fertilizer and insecticides into the water used for cultured medium. (Conyurt and Akhan, 2008).Common practices in hatcheries such as transportation, handling, cleaning, crowding, use of chemicals, and problems with water quality are stressors that may negatively influence reproduction (Billard *et al* 1995).These factors affect fertilization success in artificial reproduction commonly used for aquaculture species. As a result, low quality fish seeds are produced.

The need for high quality fish seed has necessitated research into various ways of enhancing fertility to meet the growing demand. However the continuing expansion of aquaculture requires shifting from synthetic drugs to natural plant. Medicinal plants that were once considered of no value are now being investigated, evaluated and developed into drugs with little or no side effects (Adedeji *et al.*2006b). The use of medicinal plants as fertility enhancer in aquaculture has now being receiving some attention. Dada and Ajiore (2009) used extract of *G. kola* seed to enhance fertility in *C. gariepinus*.

Kigelia africana (Lam) Benth, belongs to the family *bignoniaceae*. It is abundant in the tropics and is widely used in southern Nigeria as a herbal remedy for various ailments such as diarrhea, malaria, rheumatism, retained placenta and dizziness (Gill 1992). Sexual complaints such as infertility, poor libido, sexual asthenia and impotence are treated with medicines containing the fruits, roots or leaves of *K. africana* (Owolabi and Omogbai 2007). *K. africana* fruit extracts had been used successfully as fertility enhancing agent in rats (Abioye *et al.* 2003). It is therefore not out of place to expect a similar effect on fish. This method of enhancing fertility in fish could be easier to adopt by poor fish farmers since *K. africana* fruits are available all year round in the tropics and sub-tropical regions. Phytochemical screening of Kigelia africana showed the presence of steroid (Stahl 1988). Steroid such as androgen and estrogen have shown to contain fertility properties necessary for the improvement and production of reproductive organs (Eik *et al.* 1965). The objective of this study was to investigate the effects of varying dietary supplementation of *K. africana* on the sperm quality and fertility in *C. gariepinus*.

2. Materials and methods

2.1 Collection and Acclimatization of Experimental Fish

Experimental broodstock (mean weight, 396.05±7.04) were sourced from Agriculture Development Project (ADP), Akure, Ondo State, Nigeria. The broodstocks were conditioned for two weeks in concrete holding tanks at the department of Fisheries and aquacuture technology fish farm, Federal University of Technology, Akure, Ondo State, Nigeria. During this period they were fed with commercial diets of 40% crude protein twice daily at 3 % of their body weight.

2.2 Formulation of Experimental Diets

Fruits of the *K. africana* were collected from Ilale Keji, Village, Owo Local Government area, Ondo State, Nigeria and identified at the department of Forestry and Wood Technology, Federal University of technology, Akure, Ondo State, Nigeria. After collection, the fruits were cut into small pieces and sun dried. The sun dried fruit was grounded into fine powder and analysed for crude protein, fat, moisture, ash, crude fiber using (AOAC 1997). 50, 100, 150 and 200 g/kg *K. africana* were measured out and mixed with basal feed of 40% crude protein based on the formulation defined for African catfish *C. gariepinus* (Fagbenro and Adebayo, 2005).

Five isonitrogenous diets were formulated from practical ingredients (Table1) where the control basal diet was without *K. africana* fruit meal and the other diets were supplemented by 500, 100, 150 and 200 g/kg of *K. africana* fruit meal respectively. The experimental diets were formulated to contain almost 40 % crude protein.

Proximate composition of diet was carried out as described by AOAC (1997) (Table 1).

All dietary ingredients were weighed with a weighing top load balance (Metler Toledo, PB 8001 London). The ingredients were ground to a small particle size. Ingredients including vitamin premix and *K. africana* meal were thoroughly mixed in a Hobbart A- 2007 pelleting and mixing machine (Hobart Ltd London, England) to obtain a homogenous mass, cassava starch was added as a binder. The resultant mash was then pressed without steam through a mixer with 0.9mm die attached to the Hobart pelleting machine. The produced pellets were dried at room temperature and kept frozen until experimental start.

2.3 Experimental set up

Water was sourced from an adjacent fish pond using 1.5 HP pump and the tanks were filled to a depth of 1m. 8 female *C. gariepinus* broodstock were stocked into 15 tanks, with three replications per treatment. The diets were assigned randomly to the tanks and each group of fish was fed at 3% body weight/day in two equal portions at 900- 1000 hours and 1600-1700 hours for 90 days. All fish were removed from each concrete tank every week and batch-weighed.

2.4 Evaluation of milt quality

Milt production and quality were determined at the end of the experiment. 12 males of fish, randomly selected from each treatment, were sacrificed and the testes removed.

2.4.1 Milt Volume: Small incision was made into the lobes of the testes, the milt squeezed out into a Petri dish. This was measured with plastic syringe in ml.

2.4.2 Motility Duration: These were determined placing 1µl of milt from each male on a Neubauer hemocytometer, a drop of distilled water was added and covered with a slip. The sperm activity was viewed under Olympus microscopic at 100 x magnification to see when all the sperm got stopped (Mims 1991)

2.4.3 Percentage Motility: Each sample was estimated using light microscope at 400x magnification immediately after addition of 20 µl distilled water as an activating solution. During spermatozoa activation, immotile sperm cell (ISC) was counted, and when the activation stopped, whole sperm cells (WSC) was counted (Canyurt *et al.* 2008). The motile sperm cells (MC) was calculated as

$$MC = WSC - ISC$$

$$\% MC = MC/WSC \times 100$$

2.4.4 Milt Count: Concentration of sperm was determined by counting the number of spermatozoa in sample dilute with distilled water (100 x) in a Burker haemocytometer, under 400x magnification (Rainis *et al.* 2003).

2.5 Fertilization

This was determined when the eggs generally reached the 4-8 celled stage of embryonic development. For calculating percent fertilization of each replicate a sample was taken on a Petri dish containing water and the number of fertilized and unfertilized eggs was counted under a microscope (40 x magnification) and calculated as follows:

$$\% \text{ Fertilization} = \frac{\text{Number of fertilized eggs} \times 100}{\text{Total number of eggs counted}}$$

2.6 Water quality parameters

Water quality parameters such as temperature, pH and dissolved oxygen concentration were monitored weekly throughout the study using mercury-in-glass thermometer, pH meter (Hanna H198106 model) and dissolved oxygen meter (JPP-607 model) as described by APHA (1987).

2.7 Statistical Analysis

All values were recorded as mean ± standard deviation and subjected to one- way analysis of variance (ANOVA) using SPSS 10 for window software package. The percentage data was transformed using arcsine before statistical analysis. Significant means were subjected to a multiple comparison test (Duncan) for post hoc comparison at $\alpha = 0.05$ level.

3. Results

Table 2 shows the effect of *K. africana* meal on milt quality parameters while figure1 shows the percentage motility and fertility in *C. gariepinus*. The mean milt volume of the experimental fish ranged from 1.1 ml in diet D2 to 2.0ml in Diet D5. The highest milt volume (2.0ml) was obtained in diet D5 and the least milt volume (1.1 ml) in diet D2 and milt volume differed significantly ($P<0.05$) among the treatments. The result for motility duration showed that it ranged from 39 seconds to 51 seconds. Diet D5 was observed with highest time (51secs) and the least was recorded for Diet D3 (39secs). There was significant difference ($P<0.05$) in motility duration among the treatments. Percentage motility showed significant inter specific differences ($P<0.05$) and mean values ranged from 55% to 92%. The levels of inclusion of *K. africana* meal affects percentage motility. There was increased in percentage motility in diet D2 to diet D3 and reduction in percentage motility was observed in Diets D4 and D5. The highest value was recorded in Diet D3 (92%) and least value in diet D5 (55%). The sperm counts in the experimental groups varied between 2.6 and 6.5 x 10^9 sperm/ml. The sperm counts obtained in diets D2, D3 D4 and D5 were significantly ($P<0.05$) higher than the sperm counts in D1 (control). Diet D3 had the highest value (6.5± 1.2x10^9sperm/ml) while diet D5 had the least value (2.6±1.3x10^9sperm/ml).

4. Discussion

Viable sperm is an essential component of any successful animal production operation and the success of reproduction process is dependent on a supply of high quality gametes (Crus-Casallas *et al.* 2005). The present study confirmed that dietary inclusion of *K. africana* fruit is essential for broodstock fertility. Dietary inclusion of *K. africana* affected positively some parameters of sperm quality in *C. gariepinus*, such as sperm counts, percentage motility, milt volume and motility duration (Table 2). The inclusion resulted in weight gain of fish in diets D2 and D3 compared with control. Diet D2 showed increased in weight gain of testes when compared with control, however there were no significant

differences (P<0.05) among the treatments. These results showed that *K. africana* may have enhanced nutrient utilization which is reflected by improvement in weight gain by testes.

Similar observation of aqueous extract of *Anacyclus pyrethrum* was observed in male rats (Sharma *et al.* 2008). At present, there are no truly dependable criteria for estimating sperm quality. In human, mammals and fish, the length of time and intensity of spermatozoa motility, the percentage motile sperm and sperm density are all parameters that have been measured in an attempt to assess sperm quality (Billard and Cosson 1992). Moreover fertilizing capacity is the most conclusive way of testing sperm quality (Billard *et al.* 1995). Spermatozoa motility is the most commonly used criterion to evaluate semen quality (Bozkurt *et al.*2006), however spermatozoa motility varies in rigor and duration not only among male but also within an individual male depending on the ripeness, age and time of sampling. The highest motility of the spermatozoa is observed at the peak of the breeding season (Terner 1986). It was observed that fish fed on 100 g/kg dietary inclusion of *K. africana* showed the highest motility of 92% while diet D1 (control) had 86% motility. Investigation revealed that teleosts spermatozoa must swim actively into the micropylar channel for successful fertilization (Iwamatsu *et al.* 1993). These results agrees with Ogbeche *et al.* (2002) who reported that extract of *K.* africana had a greater sperm motility in male induced rats with 95% when compared with control having 83%. The result showed a strong relationship between motility and fertility, an increased motility resulted into increased fertility (Fig 1).

Rurangwa *et al.* (*2001*) observed a high correlation between sperm fertility and spermatozoa motility. There was significant difference (p> 0.05) in sperm counts between *C. gariepinus* fed on diet D2 and other treatments. Sperm count was higher than those reported by Steyn and Van Vuren (1987a). The authors observed 6.2 x 10^9 sperm/ml in comparison with 6.5 x 10^9 sperm/ml observed in the present study. The higher sperm count obtained in treated groups may be attributed to the presence of androgen in *K. africana* since androgen is most effective as a direct stimulator for spermatogenesis (Ogbeche *et al.* 2002). Similarly, this finding agrees with Adesanya *et al.* (2007) who reported an increased in sperm counts of rats treated with ethanol extract of *Garcinia kola.* Oluyemi *et al.* (2007) also obtained similar result with *Garcinia cambogia* while Sharma *et al.* (2008) observed increase in sperm counts with extract of *Anacyclus pyrethrum*. Poole and Dillane (1998) opined that qualitative evaluation of gametes should consider not only motility and fertility rates, but also sperm concentration. Although motility duration of 100 g/kg treatment was found to be lower among the treatments, it is expected that high progressive cell velocity might have resulted in shortage of time and there is possibility of *k. africana* boosting energy content of the cell. Kim *et al.* (1998) suggested that unknown factors in various medicinal herbs led to favourable results in fish trials. Consequently it is not necessary for sperm to have long motility duration to reach spawned eggs, as was effectively observed in this study. Fauvel *et al.* (1998) found that fertilization ability decreases exponentially with time and no fertilization occurs if sperm had been active for more than 30 sec.

During the first 20- 25 sec sperm movement shows a steady behaviour, reaching the maximal values of velocity and linearity of trajectory, in this period the probability of fertilization is the highest. The estimation of spermatozoa motility obtained in the present study is in agreement with those previously observed in the same species (Steyn and Van Vuren 1987a). In this study it was observed that milt volume increased with increased inclusion level of *K. africana*. However, diets D4 and D5 had significantly higher (p<0.05) milt volume than the control and it was observed that higher concentration *of K. africana* resulted into lower sperm counts, percentage motility and longer duration of motility. This is in agreement with Pardo-Carrasco *et al.* (2006) who evaluated the semen of *Brycon amazonicus* under induction with carp pituitary extract (CPE) and reported that volume increased without increasing the sperm counts but disagree with the findings of Adewumi *et al.* (2005) on the effect of heated soybean on sperm quality of *C. gariepinus* who reported increased in motility and sperm counts with volume of milt. Kims *et al.* (1998) stated that unknown factors in various medicinal herbs might be responsible for these occurrences. The dissolved oxygen, pH and temperature estimated during the experiment were within the acceptable range recommended for catfishes (Viveen et al. 1985).

5. Conclusion

In conclusion, dietary *K. africana* fruit meal, which improves the milt quality of cultured African catfish, *C. gariepinus* is useful and reliable method for propagating seedling production and rearing strategy. This study established the efficacy of *K. africana* fruit powder as fertility enhancer in male *C. gariepinus* broodstock and should be encouraged as it will minimize the dependence on synthetic drugs as fertility enhancing agents. Therefore, future research should focus on the improvement of seedling production technology for different fish by *K. Africana,* since the main aim of aquaculture is to maximize fish production and this plant has promising pro fertility property which can be exploited in aquaculture

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Table 1. Ingredient and proximate* composition of experimental diets

Ingredients	Experimental diets				
	D1	D2	D3	D4	D5
Fish meal (72%)	250	250	250	250	250
Yellow maize	150	150	150	150	150
Groundnut cake (45%)	260	260	260	260	260
Soybean meal (40%)	220	220	220	220	220
Vegetable oil	80	80	80	80	80
Vitamin premix**	10	10	10	10	10
Sodium chloride (Nacl)	10	10	10	10	10
Oyster shell	10	10	10	10	10
Binder (starch)	10	10	10	10	10
K. africana fruit meal(g/kg feed)	0	50	100	150	200
Proximate (% DM)*					
Moisture	9.81	11.7	10.80	10.87	10.85
Crude protein	40.01	40.64	40.95	39.88.	40.14
Crude lipid	13.85	14.00	13.46	13.83	13.16
Crude fiber	6.22	6.58	6.60	7.18	7.25
Ash	8.61	8.00	6.62	7.82	8.38
NFE***	21.50	19.08	21.57	20.42	20.22

*Determined using standard methods [AOAC 1997). All samples were analysed in triplicates.

**Vitamin premix – A Pfizer livestock product containing the following per kg of feed: A = 4500 I. U, D = 11252 I.U, E = 71 I.U, K3 = 2 mg, B12 = 0.015 mg, panthothenic acid = 5 mg, nicotinic acid = 14 mg, folic acid = 0.4 mg, biotin = 0.04 mg, choline = 150 mg, cobalt = 0.2 mg, copper = 4.5 mg, iron = 21 mg, manganese = 20 mg, iodine = 0.6 mg, selenium= 2.2 mg, zinc = 20 mg, antioxidant = 2 mg

***NFE = Nitrogen – free Extract = 100 - (Crude protein + Crude fiber + Lipid content+ Moisture content + Ash)

Table 2. Milt quality parameters of *C. gariepinus* fed dietary inclusion of *kigelia africana*

Parameters	Experimental diets				
	D1	D2	D3	D4	D5
Mean weight of fish(g)	575±205.06^{a}	610±14.14^{a}	605±134.35^{a}	465±91.92^{a}	470±42.34^{a}
Mean length of testes (mm)	9.8±1.56^{a}	8.3±3.40^{a}	10.0±2.90^{a}	8.30±1.56^{a}	9.45±3.04^{a}
Mean weight of testes (g)	5.25±1.34^{a}	5.85±1.48^{a}	4.60±0.71^{a}	4.60±2.12^{a}	4.05±0.35^{a}
Motility duration (mins)	46.50±2.12^{bc}	45.50±2.12^{b}	39.00±1.4^{a}	46.50±2.12^{bc}	51.00±1.41^{c}
Sperm count ($\times 10^{9}$ spm/ml)	293500±10606^{ab}	490000±27292^{b}	650000±2355^{c}	373500±103944^{ab}	257500±53033^{a}
Milt volume (ml)	1.75±0.71^{c}	1.1±0.00^{a}	1.45±0.71^{b}	1.8±0.14^{c}	2.0±0.14^{c}
Motility (%)	60.00±2.83^{b}	84.00±8.49^{c}	92.50±2.12^{c}	78.00±11.31b^{c}	55.00±7.07^{a}

a,b,c values (mean±S.E) with different superscripts in each line indicate significant differences ($p<0.05$)

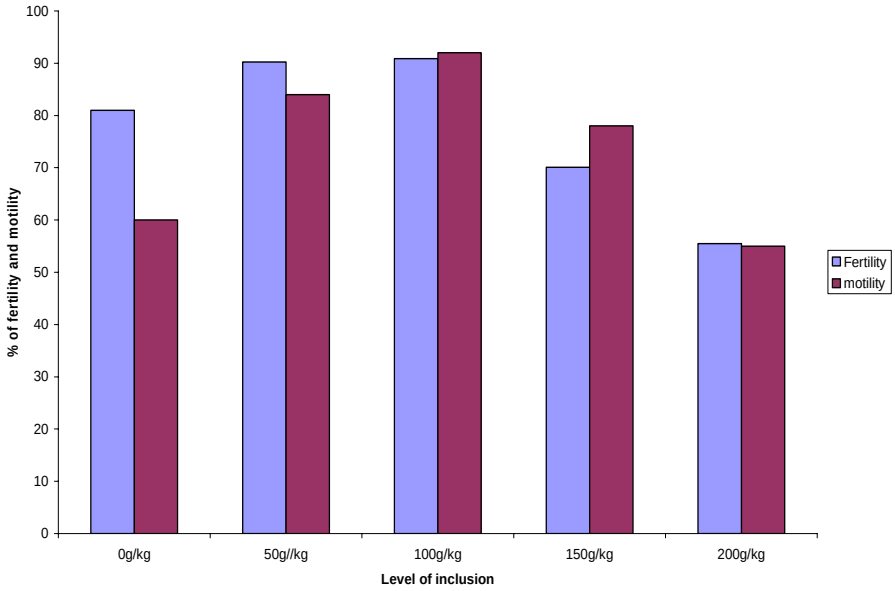

Figure 1. % fertility and motility of *C. gariepinus* broodstock

Journal of Agricultural Science
http://www.ccsenet.org/jas
Vol. 2, No. 1
March 2010

Genetic and Morpho-Agronomic Evaluation of New Tomato Breeding Lines Resistant to Bacterial Speck (*Pseudomonas Syringae* pv. *Tomato)*

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Abstract

A breeding program was initiated at the Research Institute of Vegetable Crops, Skierniewice, Poland to select new tomato lines resistant to bacterial speck (*Psedomonas syringae* pv. *tomato* (PST) and suitable for field growing. Two tomato cultivars with good agronomic characteristics Luban and Rumba were crossed with resistant to PST 'Ontario 7710'. Then backcross and pedigree breeding procedures were employed to obtain BC_5, BC_4S_1, BC_3S_2, BC_2S_3, BC_1S_4 populations. Screening test among those hybrid populations was performed in a greenhouse and exhibited a large amount of variation in response to PST infection. All plants within BC_1S_4 progeny of the 'Luban' x 'Ontario 7710' cross and two BC_1S_4, BC_3S_2 progenies of the 'Rumba' x 'Ontario 7710' cross had no symptoms of bacterial speck and could be assigned to the homozygous category. The remaining progenies were segregating for resistance and susceptibility and gave distribution of plants over all severity classes. That confirms heterozygosity for resistant gene *Pto* of their latter resistant single plant selections before backcrossing or/and selfing. The BC_5 populations had significantly higher disease severity rates (DSI = 2.4) than all other selfed populations (DSI = 1.2 – 1.6). After selection resistant genotypes of the populations were transplanted to the field for morphological traits evaluation. A noticeable progress in breeding regarding most of the plant and fruit characteristics of the recurrent parents was approached in all populations of the 'Luban' x 'Ontario 7710' cross. Although all progenies of the 'Rumba' x 'Ontario 7710' differed from their recurrent parent as to one feature at least, however the differences showed improved shifting toward the characteristics of susceptible parent.

Keywords: Lycopersicon, Bacterial speck, Morphological features, Yield quality

1. Introduction

Bacterial speck of tomato, caused by *Psedomonas syringae* pv. *tomato (*PST) occurs in all major tomato growing areas of the world. The disease attacks stems, buds, flowers, leaves and fruits, causing reduction of yield and sometimes leading to death of tomatoes plants (Louws *et al.*, 2001; Preston, 2000). In Poland, severe outbreaks of bacterial speck on tomatoes have been reported during past decade, causing lower fruit productivity and quality of cultivars (Macias, 1999). This is related to the susceptible tomato cultivars grown in Poland (Macias, 1996) and to the lack of effective chemical control of the disease (Alexander *et al.*, 1999; Jones and Jones, 1989; Ramos *et al.,* 1989; Silva and Lopes, 1995). Therefore breeding of tomatoes for genetic resistance against bacterial speck seems to be very important and promising perspective. It was found that resistance of *Lycopersicon esculentum* 'Ontario 7710' to bacterial speck is determined by a single dominant gene (Pitblado and MacNeill, 1983). Also, the evidence that this resistance is governed by semi-dominant gene was found (Kozik, 2002). However, the horticultural traits of 'Ontario 7710' are not very desirable for agronomic conditions and preferences of Polish farmers. The project was initiated to incorporate the resistance to bacterial speck into Polish cultivars with good horticultural characteristics. Newly developed breeding material has to be at least as good as, if not better than the standard cultivars in regards to their high and good quality of productivity improved by the addition of resistance to bacterial speck.

This paper includes progress information on the development of tomato breeding lines derived from crosses of susceptible cultivars Rumba and Luban with resistant to bacterial speck 'Ontario 7710'. The results obtained in 2007-2008 from evaluation of the most advanced in breeding progenies of those crosses with regard to resistance level and horticultural characteristics are also discussed.

2. Materials and methods

2.1 Plant material

Two tomato cultivars Luban and Rumba with good agronomic characteristics but susceptible to bacterial speck (PST), and 'Ontario 7710' resistant to PST and ten BC_5, BC_4S_1, BC_3S_2, BC_2S_3, BC_1S_4 populations were used in both greenhouse and field experiments.

Crosses were made between 'Rumba' and 'Luban' and the bacterial speck resistant 'Ontario 7710'. Resistant F_1 plants from each of these crosses were used in procedures of backcross and inbreeding based on a one-way program up to the Bc_5. Also after each backcross plants were selfed in consecutive generations. Each population was screened for seedling PST-resistance and only resistant seedlings were transplanted into the field for horticultural traits evaluation (such as plant vigor, fruit size, fruit weight, number of locules) or were transplanted into 10-liter plastic pots under greenhouse conditions for backcrossing or selfing. All crosses were made by hand pollination.

2.2 Screening for bacterial speck resistance (greenhouse experiments)

The bacterial strain Pst3, race 0 *Psedomonas syringae* pv. *tomato*, from the Collection of Plant Pathogens, Institute of Plant Protection, Poznan, Poland was used in all tests. This strain was found to be the least variable and the most virulent among other tested in our previous studies (Kozik and Sobiczewski, 2000). Stock cultures of bacteria were maintained on nutrient agar with 1.6% glycerol under paraffin oil at 5°C. For the preparation of inoculum, bacteria were incubated at 24°C on nutrient agar medium supplemented with 1% glucose. The inoculum was prepared by washing off 24h-old cultures of bacteria with sterile distilled water. Concentration of bacteria in the suspension was adjusted spectrophotometrically to 10^8 cfu/ml and confirmed by a serial dilution plating method. Before inoculation of tomato plants, the ability of Pst3 strain to induce hypersensitivity reaction (HR) on tobacco plants cv. Samsun was checked according to the method of Klement (1963).

Ten days after sowing the tomato seedlings were transplanted to 10 cm diameter plastic pots containing sphagnum peat substrate (Kronen Mix) and kept on benches in a greenhouse. When the plants reached the four leaf stage they were sprayed with inoculum. After inoculation the plants were kept under a plastic cover for 4 – 5 days to obtain a relative humidity of 100%. Afterwards the covers were taken out and the humidity fluctuated between 50 and 70%. The temperature set points were 27°C by day and 21ºC by night. Disease was assessed 14 days after inoculation. The lesions of bacterial speck on leaves per plant were counted and plants were classified using the scale of Chambers and Merriman (1975) as follows: 0 = no lesions; 1 = 1-10 lesions per plant; 2 = 11-20 lesions; 3 = 21-40 lesions; and 4 = more than 40 lesions per plant. Data were collected as means over all leaves on the plants within each line. Disease intensity was evaluated on the basis of disease severity index (DSI). Low DSI per genotype implies a high level of resistance and low susceptibility, while a high DSI means that resistance level is low and thus susceptibility is high (Eenink, 1981). Additionally, considering the number of spots, plants from class 0 were regarded as resistant (R), those from 1 and 2 were tolerant (T) because of the very low number of spots and lack of expansion, and those from classes 3 and 4 were considered susceptible (S), as they were typical for 'Rumba' and 'Luban'.

2.3 Morphological evaluation (field experiments)

In a second decade of May 2007 and 2008, after the selection for bacterial speck resistance, plants were transplanted to the experimental field of the Research Institute of Vegetable Crops in Skierniewice. The experiment was set up according to an experimental design with four randomized blocks with two rows of twenty plants per genotype per block. A plot size was 10 m^2 with 2.4 plants·m^{-2}. Fertilization and all other cultural practices, including irrigation in the rainfall deficiency periods, were conducted according to standard recommendations. Standard fertilizer practices were used.

Fruits were harvested once a week. Quantity of a total and marketable yield was subjected with regard to fruit fractions based on fruit diameter: marketable (>6.0 cm, 4.5-6.0 cm, 3.5-4.5 cm), nonmarketable (small = <3.5 cm, irregular, cracked, spoiled). The yield quality was recorded on the bases of the participation of marketable yield and subsequent fruit fractions in total yield in percent. Twenty fruits of each population were evaluated for morphological traits: fruit weight, number of locules and fruit shape (shape coefficient).

The share of marketable yield and other fruit fractions in total yield for parental cultivars Rumba, Luban and Ontario 7710 and their segregating crosses were similar in 2007 and 2008 and indicated that the data could be pooled. Therefore, data for each generation were pooled for the two crosses and statistical analysis were processed with STATISTICA 5.0 software. Significant differences among means were verified using Newman-Keuls' range test at alfa = 0.05.

3. Results

3.1 Variation in resistance to bacterial speck

The screening test indicated that severity of bacterial speck on all plants of the recurrent parents 'Luban' and 'Rumba' was high. Both cultivars, affected in classes 3 and 4, were assigned to the susceptible category (DSI = 3.2 and 3.5, respectively) (Table 1). It shows that applied inoculation procedure gave infection of 100% susceptible parents plants.

The donor parent 'Ontario 7710', one progeny of the Luban x Ontario 7710 cross (BC_1S_4) and two progenies of the Rumba x Ontario 7710 cross (BC_1S_4, BC_3S_2) were highly resistant with all symptomless plants within each population (DSI = 0.0). Intermediate disease severity was found in all other progenies of the Rumba and Luban x Ontario 7710 crosses. Both BC_4S_1, BC_2S_3, BC_5 and one BC_3S_2 populations showed large variation range and gave distribution of plants over all severity classes. In both BC_5 progenies a segregation ratio of 1 tolerant (classes 0, 1, 2)*:* 1 susceptible (classes 3, 4) was obtained and they had significantly higher disease severity rates (DSI = 2.4) than all other selfed populations (DSI = 1.2 – 1.6).

3.2 Variation in morphological traits

A significant progress in breeding for bacterial speck resistance in populations of cv. Luban was noticed in total yield components and fruit characteristics for most of the breeding methods used (Table 2, 3). Percentage of marketable yield, small, irregular and cracked fractions of fruits was similar to those of 'Luban'. All populations expressed lower number of diseased fruits (2.0-3.6%) than the susceptible parent (6.9%). However, there was a large variation in fruit fractions in total yield observed among populations of cv. Rumba except marketable fruits that showed similar or higher values (83.8-91.1%) to those of the susceptible parent (85.1%). Population Bc_2S_3 with respect to the most of fruit fractions in total yield were approaching to those of 'Rumba'. The best results were recorded for the most advanced in backcrossing populations BC_4S_1 and BC_5 where percentage of irregular and diseased fruits was lower than in their susceptible parent 'Rumba' (Table 2).

Data on morphological traits of fruits revealed no significant differences between 'Luban' and its consecutive populations (Table 3). Fruit weight, shape coefficient, number of locules and fruit hardness for all tested progeny of the 'Luban' x 'Ontario 7710' cross were similar to those of susceptible parent. In contrast to the populations of cv. Luban, progenies of the 'Rumba' x 'Ontario 7710' cross constituted a more diversified group. The most advanced in backcrossing populations BC_5 and BC_4S_1 had the most lighter fruit (91.9 and 94.8g, respectively), typical for 'Rumba'. In remaining populations, more heavy than for susceptible parent fruit persisted. All tested populations of the 'Rumba' x 'Ontario 7710' cross were characterized by higher fruit hardness than their recurrent parent (Table 3).

4. Discussion

Previous studies have shown that resistance to bacterial speck (*Psedomonas syringae* pv. *tomato*) (PST) in 'Ontario 7710' is controlled by a single dominant gene (Pilovsky and Zutra, 1982) or semi-dominant gene (Carland and Staskawicz, 1993; Kozik, 2002). Backcross pedigree breeding program and selection for bacterial speck resistance was required to maintain resistance and to obtain breeding lines with uniform resistance and good plant and fruit characteristics typical for both susceptible recurrent parent "Rumba' and Luban'. Data in this study revealed that plants that carry resistant gene *Pto* to bacterial speck can be found in all of the tested populations, but genetic backgrounds of the families were different and depended on the homo/heterozygous status of resistant gene *Pto*. All plants within BC_1S_4 progeny of the 'Luban' x 'Ontario 7710' cross and two BC_1S_4, BC_3S_2 progenies of the 'Rumba' x 'Ontario 7710' cross had no symptoms of PST. These three progenies were uniformly resistant and could be assigned to the homozygous category. That also suggests that the previous single plant selection, before selfing, was homozygous for resistant gene *Pto*. The remaining progenies were segregating for resistance and susceptibility that confirms heterozygosity for resistant gene *Pto* of their latter resistant single plant selections before backcrossing or/and selfing. Presumably it may take one or two selfings to obtain other breeding lines with uniform, stable resistance to bacterial speck in case of more advanced in breeding populations and three or more selfings in case of two backcross populations BC_5.

Generally, if backcross breeding is to be successful, the genotype of the recurrent parent must be recovered in its essential plant and fruit features. This is primarily a function of the number of backcrosses, although selection for the type of the recurrent parent in the early backcross generations is effective in shifting the population towards the characteristics of that parent. It is believed that six backcrosses coupled with rigid selection in early generations produce plants that resemble the recurrent parent effectively (Allard, 1960). Processing tomato lines resistant to bacterial speck, developed in a backcross program employing tomato cv. UC 82 as the recurrent parent and the accession Ontario 7710 as the donor of the *Pto* resistance gene were reported earlier (Candilo and Calzolari, 1992). Backcross lines exhibited a resistance similar to the donor parent and significantly higher than the recurrent parent. Several lines achieved marketable yield similar or superior to those of cultivar UC 82. In our study a noticeable progress in breeding regarding most of the plant and fruit characteristics of the recurrent parents was approached in all populations of the 'Luban' x 'Ontario 7710' cross. Although all progenies of the 'Rumba' x 'Ontario 7710' differed from their recurrent parent as to one feature at least, however the differences showed improved shifting toward the characteristics of susceptible parent. The results also revealed that backcross pedigree programs coupled with a particularly high intensity of selection for bacterial speck resistance and the type of recurrent parent made variation among methods insignificant.

5. Conclusions

Three of 10 developed progenies of 'Luban' x 'Ontario 7710' cross (BC_1S_4) and 'Rumba' x 'Ontario 7710' cross (BC_1S_4, BC_3S_2) were uniformly resistant to bacterial speck and could be assigned to the homozygous category.

Resistant plants with good horticultural characteristics were obtained in all seven remaining generations that segregated in resistance responses to bacterial speck.

Several additional selfs will be required to develop other bacterial speck resistant breeding lines with uniform resistance and good horticultural characteristics.

A noticeable progress in breeding regarding most of the plant and fruit characteristics of the recurrent parents was approached in all populations of the 'Luban' x 'Ontario 7710' cross.

Although all progenies of the 'Rumba' x 'Ontario 7710' differed from their recurrent parent as to one feature at least, however the differences showed improved shifting toward the characteristics of susceptible parent.

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Table 1. Distribution of seedlings of parents and progenies from crosses between the bacterial speck resistant 'Ontario 7710' and susceptible cvs. Luban and Rumba by disease rating

Population	No. of plants in infection classes					Total number of tested plants	DSI*
	0	1	2	3	4		
Luban				24	6	30	3.2 d
Bc_5	6	14	10	12	18	60	2.4 c
Bc_4S_1	52	24	10	8	24	118	1.4 b
Bc_3S_2	32	34	32	22		120	1.4 b
Bc_2S_3	86	54	28	46	18	232	1.4 b
Bc_1S_4	120					120	0.0 a
Rumba				16	14	30	3.5 d
Bc_5	4	13	13	13	17	60	2.4 c
Bc_4S_1	46	16	16	24	18	120	1.6 b
Bc_3S_2	120					120	0.0 a
Bc_2S_3	48	26	4	28	2	108	1.2 b
Bc_1S_4	120					120	0.0 a
Ontario 7710	30					30	0.0 a

*DSI = disease severity index,

Means followed by the same letter are not significantly different at alfa = 0.05

Table 2. Yield components in populations from a cross between the bacterial speck resistant 'Ontario 7710' and susceptible cvs. Luban and Rumba

Population	Fruit fractions in total yield in %				
	marketable yield	nonmarketable yield			
		small <3.5cm	irregular	cracked	spoiled
Luban	**92.3 b**	**0.5 g**	**0.2 g**	**0.0**	**6.9 b**
Bc_5	95.2 a	1.2 e	0.0 h	0.0	3.6 cd
Bc_4S_1	95.6 a	1.0 e	1.0 d	0.0	2.5 de
Bc_3S_2	95.2 a	1.2 e	1.7 c	0.0	2.0 e
Bc_2S_3	94.4 a	0.6 fg	1.8 c	0.0	3.3 cd
Bc_1S_4	95.4 ab	0.8 e-g	0.8 e	0.0	2.9 c-e
Rumba	**85.4 d**	**3.4 c**	**2.2 b**	**1.0**	**8.0 a**
Bc_5	88.2 c	2.9 d	0.6 f	0.0	6.2 b
Bc_4S_1	88.5 c	4.2 b	0.5 f	0.0	6.9 b
Bc_3S_2	83.8 d	6.3 a	2.2 b	0.0	7.8 ab
Bc_2S_3	91.1 b	3.2 c	1.7 c	0.1	3.9 c
Bc_1S_4	90.5 b	0.9 fg	4.1 a	0.0	4.2 c
Ontario 7710	**88.5 c**	**2.6 d**	**0.0 h**	**0.0**	**8.8 a**

Means followed by the same letter are not significantly different at alfa = 0.05

Table 3. Fruit characteristics in populations from a cross between the bacterial speck resistant 'Ontario 7710' and susceptible cvs. Luban and Rumba

Population	Weight of marketable fruit (g)	Shape coefficient	Number of locules	Hardness*
Luban	**107.7**	**0.8**	**4.7**	**2.6**
Bc_5	102.3	0.8	4.6	2.9
Bc_4S_1	101.5	0.8	4.7	2.8
Bc_3S_2	103.1	0.8	4.6	2.8
Bc_2S_3	112.3	0.9	4.7	2.6
Bc_1S_4	102.9	0.9	4.4	2.9
Rumba	**92.1**	**0.8**	**4.9**	**1.9**
Bc_5	91.9	0.8	4.4	3.3
Bc_4S_1	94.8	0.9	4.2	2.6
Bc_3S_2	104.4	0.8	5.0	3.0
Bc_2S_3	100.4	0.8	4.2	2.6
Bc_1S_4	105.9	0.9	4.4	2.6
Ontario 7710	**45.6**	**1.2**	**2.2**	**3.4**

***** hardness after 2 weeks of storage on a 1-5 scale with 1 = soft fruits, 3 = medium hard, 5 = hard fruits

Means followed by the same letter are not significantly different at alfa = 0.05

Journal of Agricultural Science
http://www.ccsenet.org/jas
Vol. 2, No. 1
March 2010

An Evaluation of Groundnut Processing by Women in a Rural Area of North Central Nigeria

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Abstract

This study evaluated the economic empowerment potentials of groundnut processing by women in rural areas of North central Nigeria state using a sample of 100 women processors randomly selected from the study area. Data analysis was done using Descriptive statistics, Net Farm Income Model and Data Envelopment Analysis (D.E.A). An average net returns of **N**10, 586.6 was obtainable within a processing cycle. The average pure technical and scale efficiency scores were 80 and 83 percent respectively. The major constraints confronting the processing of groundnut include inadequate capital for expansion and lack of processing machines. A significant opportunity exists for empowering rural women through groundnut processing.

Keywords: Groundnut, Processing, Women, Technical efficiency, Scale efficiency

1. Introduction

Groundnut (*Arachis hypogea L.)* otherwise called peanut, monkey nut, gobber pea and *arachide* belongs to the family *leguminosea*. It originated from Latin America and the Portuguese who were responsible for its introduction into West Africa from Brazil in the 16th Century (Gibbon and Pain, 1985; Abalu and Etuk, 1986). Peanut is one of the most popular commercial crops in Nigeria. Nigeria produces 41% of the total groundnut production in West Africa (Echekwu and Emeka, 2005). It is cultivated for its kernels, the oil and hay for livestock. Groundnut cake is often deep fired or dried to make a snack locally called *kuli-kuli*. Groundnut flour is used as an ingredient in soups, sweet, confectionaries and puddings. Groundnut especially those produced in developing countries has been used traditionally since the origin of humanity. It is rich in oil and protein and has a high energy value.

Developing countries account for nearly 95 percent of world production (Echekwu and Emeka, 2005). Asia accounts for about 70% of this amount while the major producers, India and China together represent over two-thirds of global output. Other important producers of groundnut are: Nigeria, Senegal, Sudan and Argentina. Groundnut with 25% protein and more than 40% oil, is an important food crop in many areas of semi-arid tropics (Food and Agriculture Organisation, 1994).

In Nigeria, the processing of groundnut into various products is mostly done by women either for home consumption or for commercial purposes (Ibrahim *et al*., 2005). The most common commercial products of groundnut are: groundnut oil, groundnut cake and fried peanuts which are sold at market places or hawked on the streets, (Ihekoronye and Ngoddy, 1985). The processing of groundnut is both the source of income and employment to a large proportion of rural women in northern Nigeria. Thus, the achievement of the Millennium Development Goal number three (promotion of gender equality and women empowerment) in northern Nigeria, requires that a study be conducted to assess the economic

empowerment potentials of this very important economic activity. In addition, the technical and scale efficiency in groundnut processing were also determined alongside the constraints affecting the processing of groundnut by rural women.

2. Issues in literature on Data Envelopment Analysis

Data Envelopment Analysis (DEA) is a non-parametric, linear programming based frontier analysis method that was originally developed to analyze the performance of organizations whose goals are not limited to profit maximization (Charnes *et al*., 1978). Data Envelopment Analysis (DEA) uses a non-parametric non stochastic piecewise linear production frontier in estimating technical efficiency. The DEA frontier estimates efficiency relative to the Pareto-efficient frontier which estimates best performance. Furthermore, it can obtain target values based on the best practices units (peers) for each inefficient firm that can be used to provide guidelines for improved performance (Abdulwadud, 2000).

The technique (DEA) is flexible in that it does not require specification of an underlying production relationship between inputs and outputs. It is able to incorporate inputs and outputs that are measured in different units and at different scales, and can accommodate multiple inputs and multiple outputs with minimal value judgments placed on the relative "worth" or "cost" of these inputs and outputs (Frija *et al.,* 2008). According to Diaz *et al* (2004), a DEA model may be either input-oriented or output-oriented. As such, in deciding on the orientation of a DEA model one should also consider over which variables decision making units (DMUs) have most control (a sample of producers are referred to as decision making units (DMU) in DEA terminology). If DMUs have more control over output variables than input variables, the DEA model should be output-oriented; otherwise, the model should be input-oriented.

Furthermore, both output-oriented and input-oriented DEA models produce the same technical efficiency estimate for a farm under the assumption of constant returns to scale in production (Lovell, 1993). Under the assumption of variable returns to scale, the estimates of technical efficiency will differ. However, Coelli (1995) claims that since linear programming does not suffer from statistical problems such as simultaneous equation bias, the choice of a measure does not affect the efficiency estimates significantly.

Since none of the production frontier models used in empirical analyses of production efficiency is without its limitations, it is very important to make a careful choice of model. Coelli (1995) identified some weaknesses of the technique as follows.

(1) Since DEA is an extreme point technique, noise (even symmetrical noise with zero mean) such as measurement error can cause significant problems.

(2) A standard formulation of DEA creates a separate linear program for each DMU, thus large problems can be computationally intensive.

(3) DEA is good at estimating "relative" efficiency of a DMU but it converges very slowly to "absolute" efficiency. In other words, it can tell you how well you are doing compared to your peers but not compared to a "theoretical maximum."

(4) The main criticism of deterministic frontiers is that they rule out the possibility of a deviation from the frontier being caused by measurement error or other noise (such as bad weather). Therefore, any deviations from the estimated frontier are attributed to inefficiency.

Econometric stochastic production frontiers, however, obviate these criticisms. Furthermore, they provide a measure of the reliability of the technical efficiency estimates by means of the standard errors of the model parameters. However, this benefit comes at the cost of imposing assumptions about the functional form of the production technology and the distribution of the inefficiency term. These assumptions affect the analysis and distort efficiency scores (Fraser and Cordina, 1999). Avoiding such assumptions is an advantage of the DEA approach (Jafarullah and Premachandra, 2003). The minimum assumption DEA requires is the monotonicity and convexity of the efficient frontier (Abdulwadud, 2000).

The major weakness of DEA relates to its inability to account for measurement error (Kalyan, 2002). However, Banker (1996) and Fare and Grosskopf (1995) proposed several statistical tests which have subsequently made DEA a powerful tool for efficiency analysis Despite its limitations, DEA is surely a competitor with the stochastic production frontier in efficiency analysis. Several researchers such as Dalton (2004), Reig-Martinez and Picazo-Tadeo (2004), Abdulwadud (2000), Ogunyinka *et al* (2004) and Helfand (2003) have used DEA for estimating technical efficiency in agriculture.

3. Materials and methods

The study was conducted in five rural areas spread across the north central zone Nigeria. The locations are largely agrarian with the majority of the people as subsistence farmers who cultivate crops such as groundnut, yam, maize, sesame, cassava, cowpea, millet, and sorghum. Twenty women groundnut processors were randomly selected from each location to give a total of 100 respondents for the study. Data was collected with the aid of an interview schedule. Data

was collected on the socio-economic characteristics of groundnut processors as well as input such as; raw groundnut, capital, machines, labour, and the outputs. The data collected were analyzed using simple descriptive statistics, Net farm income model and Data Envelopment Analysis. The Net farm income model is expressed as:

NFI = TR (Qc x Pc + Qo x Pou) – TC (TVC + TFC)

Where: - NFI = Net farm income

TR = Total revenue (from cake and oil)

Qc = Quantity of cake

Pc = Price of cake

Qo = Quantity of oil

Pou = Price of oil per unit

TC = Total cost

TVC = Total Variable cost

TFC = Total fixed cost

The variable cost items considered include capital (cost of transportation, firewood, and packaging), labour, cost of grinding, water, salt and raw groundnut. The fixed cost items include; drums, basin, processing machine, frying pan and mortar.

3.1 Data Envelopment Analysis (DEA)

Data Envelopment Analysis is a non-parametric, linear programming based frontier analysis method that was originally developed to analyze the performance of organizations whose goals are not limited to profit maximization (Charnes *et al*., 1978). Data Envelopment Analysis (DEA) uses a non-parametric non stochastic piecewise linear production frontier in estimating technical efficiency. The DEA frontier estimates efficiency relative to the pareto-efficiency frontier which estimates best performance. An output-oriented variable returns to scale DEA model was used to calculate technical, and scale efficiency in groundnut processing. The output oriented model estimates the proportional increase in outputs as inputs remains the unchanged. Assuming that there is data available on K inputs and M outputs in each of the N decision making units (i.e. processing) and input and output vectors are represented by the vectors x and y, respectively for the ith processor. The data for all processors may be denoted by the K N input matrix (X) and M N output matrix (Y). The envelope form of input-oriented VRS DEA model which is the most widely used is then specified according to Coelli, *et al* (1998) and Sharma *et al* (1999) as follows: Min $\theta\lambda$ θ

St $-y_1 + Y\lambda \geq 0$

$\theta x_1 - X\lambda \geq 0$

$NI'\lambda = 1$

$\lambda \geq 0$

Where λ is scalar and is a N x 1 vector of constraints, the value of θ obtained signifies the efficiency score for the ith DMU. It will satisfy $\theta \leq 1$with a value of 1 indicating a point on the frontier hence a technically efficient DMU according to Farrell (1957) definition. Thus, the linear programming problem needs to be solved N times and a value of θ is provided for each the processor (DMU) in the sample. Both CRS and the VRS DEA are conducted on the same data set and the ratio between the CRS and the VRS technical efficiency scores ($CRS^{T.E}/VRS^{T.E}$) is called scale efficiency (Latruffe *et al*, 2005). Efficiency scores in the study were estimated using the computer program, DEAP version 2.1 described in Coelli (1996). The inputs considered include: Raw groundnut (kg), Water (litres), Labour (man/days), Salt (g), Capital (firewood, packaging and transportation). The outputs considered include: Oil (litres) and Cake (kg).

4. Results and Discussion

4.1 Inputs and outputs in groundnut processing

The result shows inputs used and outputs obtained in groundnut processing. The inputs used include raw groundnut, water, salt and firewood. Others include fuel (kerosene) and labour. In a processing cycle of about 4 days, the total quantity of groundnut processed was 3862.80kg with an average of 154.5120kg. The total quantity of water used was 1160.00 litres with an average of 46.400 litres per processor while the total quantity of fuel (kerosene) used was 44.00 litres. Furthermore, Table 1 shows the total of groundnut cake obtained was 2236.80kg with an average of 89.4720kg per processor while the total quantity of groundnut oil obtained was 1520.00 litres with an average of 60.800 litres.

4.2 Costs and returns analysis in groundnut processing

The result for the cost and returns analysis is presented in Table 2. The average total cost of processing was N20,250.9,

which was dominated by the variable cost of processing which accounted for 90.7% of the average total cost. The fixed cost component on the other hand accounts for 9.3% of the average total cost of processing. The cost of raw groundnut dominated the variable cost by accounting for 79.59 of the total variable cost.

In terms of returns, an average gross returns of N30,817.6 per processing cycle was obtained from groundnut processing. The average gross returns was dominated by the return from groundnut oil which accounted for 56.3% of the average gross returns while the groundnut cake (*kuli-kuli*) accounts for 43.7% of the average gross returns. The revenue from groundnut oil also accounts for 85.5% of the total average cost of processing. This implies that for the processors to make sufficient profit, they have to sell both groundnut cake and groundnut oil. A similar finding was made by Hamidu, *et al* (2007). The result further shows that the average net return of N10,586.6 per processing cycle of about four days was obtained in groundnut processing by rural women in the study area. This means that in a month, net revenue of about N74106.20 was obtainable.

4.3 Pure Technical Efficiency in Groundnut Processing

An improvement in technical efficiency is essential for enhancing the profitability of any enterprise. An assessment of the level of technical efficiency in groundnut processing was done to provide further insights into the nature and causes of inefficiency in groundnut processing. The technical efficiency in groundnut processing in the study area varies from 0.07% for the 'least' practice processors and 100% for the 'best' practice processors with a mean value of 0.802. Thus, in the short run, there is scope for increasing the outputs of groundnut oil/cake by about 20 percent through improvement in technical efficiency.

4.4 Scale Efficiency in Groundnut Processing

The scale Efficiency in groundnut processing in the study area varies from 12.4% to 100% with a mean of 83%. This implies that, the groundnut processors in the study area need to increase their scale of operation by 17% to attain full scale efficiency. If the average scale efficiency score is less than the average pure technical efficiency score, then scale inefficiency is the cause of overall inefficiency (Krasachat, 2003). Otherwise, it is attributed to inefficient management practices (Latruffe *et al*., 2005). Hence, the low average pure technical efficiency (80 percent) in comparison to the average scale efficiency (83 percent) as shown in Table 3 and 4 respectively. Implies that pure Technical Efficiency in the cause of overall inefficiency. This implies that inefficiency in groundnut processing is due to managerial factors and not the scale of operation.

4.5 The constraints faced by rural women in groundnut processing

The constraints militating against groundnut processing in the study area varies from one respondent to another. However, ten constraints were identified as shown in Table 5. The processors pointed out that inadequate capital for expansion, unstable price of inputs and inadequate processing machines are the three major constraints hindering the processing of groundnut. A similar finding was also made by Haruna *et al.*, 2006). The respondents also pointed out that their profit will increase if the constraints can be overcome.

5. Conclusion

Inefficiency in groundnut processing is due to managerial factors and not the scale of operation. Furthermore, a significant opportunity exists for empowering rural women and alleviating poverty in north central Nigeria through groundnut processing. This opportunity can be exploited through improvement in managerial ability and provision of advisory services.

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Table 1. Inputs and outputs in groundnut processing

N	MINIMUM	MAXIMUM	TOTAL	MEAN
Groundnut oil	30.00	120.00	1520.00	60.8000
Groundnut cake	37.60	112.80	2236.80	154.5120
Raw Groundnut	90.00	180.00	3862.80	154.5120
Water	20.00	80.00	1160.00	46.4000
Salt	30	25	38.15	1.5260
Firewood (bundles)	1.00	12.00	98.00	3.9200
Fuel (kerosene)	0.00	20.00	44.00	1.7600
Labour	0.38	1.20	21.03	0.8412

Table 2. Cost and returns analysis from groundnut processing (US $ = **N** 153:00)

COST/RETURNS COMPONENTS	UNIT	QUANTITY	COST PER UNIT	COST (N)	%
A. VARIABLE COST	N				
i. Raw groundnut	Kg	94.6	170	16,080	79.5%
ii. Water	Litres	45.6	1	45.6	0.2%
iii. Salt	Kg	1.5	50	78.0	0.4%
iv. Firewood	Bundle	3.5	200	712	3.5%
v. Fuel (kerosene)	Litres	0.83	200	166	0.8%
vi. Labour	Manday	1.2368	250	3092	1.5%
vii. Other cost (transport, extracting oil, Grinding and marketing charges	N	-	-	952.8	4.7%
TOTAL VARIABLE COST	N	-	-	18.363.6	90.7%
B. FIXED COST					
Depreciation/Repair/maintenance	N	-	-	1,887.3	9.3
C. TOTAL COST (TVC+FC)	N	-	-	20,250.9	100%
D. REVENUE:					
i. Revenue from groundnut cake	N	5.2	2,600	13.512.8	43.8%
ii. Revenue from groundnut oil	N	2.8	6,200	17,304.8	56.2%
E. GROSS REVENUE (D_i+D_{ii})	N			30,817.6	100%
F. NET RETURN (E-C)	N			10,586.6	-

Table 3. Pure technical efficiency estimates in groundnut processing

CLASS INTERVAL	FREQUENCY	PERCENTAGE (%)
0.071 – 0.2568	9	9%
0.2569 – 0.4426	11	11%
0.4427 – 0.6284	6	6%
0.6285 – 0.8142	8	8%
0.8143 – 1.0	66	66%
Total	ΣF = 100	100%
Minimum Technical Efficiency = 0.071 Maximum Technical Efficiency = 1.0 Mean = 0.802		

Table 4. Scale efficiency estimates in groundnut processing

CLASS INTERVAL	FREQUENCY	PERCENTAGE (%)
0.124 – 0.2992	8	8.%
0.2993 - 04744	10	10%
0.4745 – 0.6496	1	1%
0.6497 – 0.8248	14	14%
0.8249 – 1.0	67	67%
Total	ΣF = 100	100%
Minimum scale Efficiency = 0.124 Maximum Scale Efficiency = 1.0i Mean = 0.827		

Table 5. Constraints faced by women groundnut processors

CONSTRAINTS	FREQUENCY	PERCENTAGE	RANKING
In adequate labour supply	08	2.1.0	10
Inadequate capital for expansion	77	21.0	1
Unstable price of inputs	74	20.0	2
Unstable price of outputs	27	7.2	6
Lack of readily available market	16	4.3	7
Incomplete return of credit sales	13	3.4	8
Low volume of production	49	13.0	4
Inadequate Processing machines	60	16.0	3
Unstable Electricity	11	3.0	9
Lack of processing shed	38	10.0	5
TOTAL	**373***	**100**	

***Multiple responses allowed.**

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E-mail jas@ccsenet.org
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Printer Paintsky Printing Inc.
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ISSN 1916-9752