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Crossability Studies among Twenty Accessions of Roselle (*Hibiscus sabdariffa* L.)

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Abstract

Increasing exploitation of roselle (*Hibiscus sabdariffa* L.) and its products require that commercial varieties with superior attributes are developed to replace landrace varieties and to meet the growing demand for commercial-scale cultivation of the crop. In this study, twenty landrace varieties of roselle collected from three geographical regions of Ghana were artificially crossed in a pairwise mating scheme. The aim was to obtain relevant information pertaining to fertility status and extent of crossability among the accessions to facilitate breeding of improved varieties. Attempts at hybridisation were made both between and among 6 accessions of *Hibiscus sabdariffa* var Altissima and 14 accessions of *Hibiscus sabdariffa* var Sabdariffa. Crosses between accessions of *Hibiscus sabdariffa* var Altissima and *Hibiscus sabdariffa* var Sabdariffa yielded no hybrids, suggestive of a crossability barrier between the two mating groups. All intra-varietal crosses involving accessions of *Hibiscus sabdariffa* var Altissima were successful regardless of direction of cross while only few of the crosses among those of *Hibiscus sabdariffa* var Sabdariffa produced successful hybrids. Also fertility estimates of members of *Hibiscus sabdariffa* var Altissima were significantly higher (30.00%-80.00%) compared to those of var Sabdariffa (0.00%-43.00%). This implies that complete pairwise design could be used for intra-varietal crosses involving accessions of *Hibiscus sabdariffa* var Altissima, whereas only a partial pairwise design is feasible for members of *Hibiscus sabdariffa* var Sabdariffa.

Keywords: crossability studies, *Hibiscus sabdariffa* L., crosses, accessions, roselle, vegetables

1. Introduction

Roselle (*Hibiscus sabdariffa* L.) is an important multipurpose crop grown in tropical and sub-tropical regions of the world for its stem fibre, edible calyces, leaves and seeds (Siti et al., 2017). It possesses huge potential as a crop of strategic importance for agricultural economies across West Africa due to its diverse uses as food (vegetables, source of oil, refreshing drink), medicine and a source of foreign exchange (fibre and natural colourants). It is rich in essential nutrients, particularly protein and iron whose deficiency is widespread especially among rural communities across the West African sub-region (Zaman et al., 2017; Chun-Tang et al., 2015).

Indeed, roselle ranks higher in nutrients and phytochemical contents than most cultivated crops in the tropics (Zaman et al., 2017; FAO, 2007). Additionally, a kind of non-alcoholic beverage obtained from the dry calyces known variously as ‘‘Bissap’’ or ‘‘Sobolobo’’ (Ghana) has gained popularity in recent years forming prominent part of refreshment packages served at various social gatherings throughout West Africa (Singh et al., 2017). Besides, genes for resistance to Yellow leaf mosaic virus infection whose damaging effect is ubiquitous among crops of the malvaceae family is reported to have been found in roselle germplasm (Chatterjee et al., 2005).

Very little effort has been directed to combine its outstanding nutritional qualities with other agronomically desirable traits in order to develop superior varieties for cultivation by farmers. Consequently, existing cultivars are wild extractions characterised by several undesirable traits such as conspicuous pubescence on almost every part of the crop, indeterminate flowering and fruiting habits as well as susceptibility to numerous diseases and

pests. The presence of these traits has made roselle an unattractive crop for commercial cultivation by farmers. Although it is adapted to a wide range of agro-ecologies, it is currently cultivated on the least preferred parcels of land, often as a relay crop, thereby portraying it as a minor crop (Patel, 2014).

Concerted efforts are therefore required to reverse this trend beginning with hybridisation among existing cultivars to generate segregating populations, followed by recurrent selection of new varieties with combined superior attributes. However, a previous hybridisation study suggests possible crossability barrier among genotypes of roselle (Vaidya, 2000). A study of crossability is therefore crucial to provide relevant information on compatibility and fertility status of the various varieties of roselle cultivated in Ghana as potential sources of breeding materials for improvement of the crop. Knowledge of compatibility of available local cultivars would enable breeders to select appropriate mating design to accelerate improvement of the crop through breeding.

2. Materials and Methods

2.1 Site of Experiment

The study was undertaken at the research farm of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC) at Kwabenya, Accra. The site is located at 05º40′ N and longitude 0º13′ W with an elevation of 76 m above sea level within the coastal savannah agro-ecological zone of Ghana. The experiment was carried out from May, 2013 to November, 2013.

2.2 Germplasm Assembly

Twenty local accessions of roselle (*H. sabdariffa*); comprising 6 cultivars of *Hibiscus sabdariffa* var Altissima and 14 cultivars of *Hibiscus sabdariffa* var Sabdariffa collected from three geographic regions of Ghana (Northern, Volta and Western) were used as parents to produce F1 hybrids (Table 1).

Table 1. Identities and Geographic location of 20 Accessions used for the study

Number	Accession	Sub-species	Geographic Region
1	A1	*Hibiscus sabdariffa* var Sabdariffa	Northern
2	A2	"	Northern
3	A3	"	Volta
4	A4	"	Volta
5	A5	"	Volta
6	A6	"	Volta
7	A7	"	Northern
8	A8	"	Volta
9	A9	"	Western
10	A10	"	Volta
11	A11	"	Western
12	A12	"	Western
13	A13	"	Western
14	A14	"	Western
15	D1	*Hibiscus sabdariffa* var Altissima	Northern
16	D2	"	Northern
17	D3	"	Northern
18	D4	"	Northern
19	D5	"	Northern
20	D6	"	Northern

This sampling was done in order to obtain relevant information on compatibility and fertility status of the various varieties of roselle cultivated in Ghana for utilisation in further breeding work.

2.3 Field Planting

Seeds of each accession were sown manually on the field in a row of ten stands separated at a distance of 60 cm between rows and 30 cm within rows. Five seeds of each accession were initially sown and later thinned to two plants per stand at two weeks after emergence. Prior to sowing of seeds, the vegetation cover of the field was

cleared and ploughing was undertaken to loosen the soil. Seeds of each accession were also planted in pots filled with potting medium comprising three parts of soil to one part of manure. The plants sown in pots were also thinned to two plants per pot at two weeks after emergence. Watering was done twice daily throughout the period. No fertiliser was applied.

2.4 Emasculation and Pollination

At flowering, ten healthy plants of each accession comprising five plants from the field and five from the pots were selected to serve as parents for hybridisation. Emasculation was carried out prior to pollination, by carefully removing anthers from matured buds which were due to open the next day using a fine-tip forceps to prevent self-pollination. Between successive emasculation of flowers belonging to different accessions, the pair of forceps was sterilised with alcohol to prevent contamination with pollen from earlier sources

Pollination was performed by dusting the stigma of the emasculated flower with pollen from a selected male parent. For each cross, thirty flowers were pollinated and reciprocal crosses were also made from 6:00 am to 10:00 am over a period of 8 weeks. Immediately after emasculation and pollination, the flower buds were covered with paper bags and clipped to prevent contamination from undesirable pollen and tagged with a label in order to identify the pollen donor and recipient as well as the time at which each particular cross was made.

2.5 Evaluation of Hybridisation Success

Compatibility was assessed through observation of each flower up to two weeks after pollination. Fertilised flowers developed fruit capsules with seeds between 7 to 14 days after pollination depending on the accessions involved in the cross. Where fertilisation failed the flowers dropped 2 to 3 days after pollination.

Hybridisation success of each cross was calculated according to the formula of Nunekpeku et al. (2012):

$$\text{Hybridisation success (\%)} = \frac{\text{Number of fruits formed}}{\text{Number of flowers pollinated}} \times 100\% \quad (1)$$

Data obtained from the plants planted in the field and those maintained in pots were combined to calculate the values for the hybridisation success.

3. Results

3.1 Cross Compatibility Estimates among Accessions of Hibiscus sabdariffa var Altissima and Hibiscus sabdariffa var Sabdariffa

No hybrids were recorded from inter-varietal crosses between accessions of var Altissima and var Sabdariffa in this study (Table 2). All attempts at hybridisation across the two crossing groups failed to develop fruit capsules as flowers shriveled and abscised two or three days after pollination.

Table 2. Estimates of inter-varietal crossability between accessions of *Hibiscus sabdariffa* var Altissima and *Hibiscus sabdariffa* var Sabdariffa

Cross	**Number of crosses**			**Compatibility**	**Comment**
Female/Male	**Attempted**	**Successful**	**Unsuccessful**		
A1 × D2	30	0	30	No	No fruits formed
D2 × A1	30	0	30	No	No fruits formed
A3 × D6	30	0	30	No	No fruits formed
D6 × A3	30	0	30	No	No fruits formed
A9 × D1	30	0	30	No	No fruits formed
D1 × A9	30	0	30	No	No fruits formed
A2 × D5	30	0	30	No	No fruits formed
D5 × A2	30	0	30	No	No fruits formed
A11 × D3	30	0	30	No	No fruits formed
D3 × A11	30	0	30	No	No fruits formed

However, hybrids were obtained from intra-varietal crosses among accessions of both *Hibiscus sabdariffa* var Altissima and *Hibiscus sabdariffa* var Sabdariffa although to varying degrees in different cross combinations. All direct and reciprocal crosses among accessions of *Hibiscus sabdariffa* var Altissima were successful (Table 3). Also, average crossability estimates among accessions in this group were very close ranging from 46.67% to 55.33 (where each accessions was repeatedly crossed as female parent to the other accessions) and 42.67% to

55.33% in reciprocal direction. The cross between accession D3 (female) and D4 (male) scored the highest success rate (80%) among the individual cross combinations in this group while D1 × D5, D3 × D1, and D6 × D1 recorded the least value (30%).

Table 3. Intra-varietal crossability estimates among six accessions of *Hibiscus sabdariffa* var Altissima

Female parent (♀)	**Male parent (♂)**						**Mean**
Accession	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	
D1		63.30	50.00	40.00	30.00	56.67	48.00
D2	73.33		46.67	36.67	63.33	56.67	55.33
D3	30.00	46.67		**80.00**	41.94	40.00	48.00
D4	36.67	50.00	60.00		33.33	53.33	46.67
D5	43.33	40.00	53.33	50.00		50.00	47.33
D6	30.00	76.67	50.00	50.00	43.33		50.00
Mean	**42.67**	**55.33**	**54.67**	**53.33**	**42.67**	**51.33**	

Note. Bolded and underlined values represent highest and least values, respectively.

Most cross combinations attempted among accessions of *Hibiscus sabdariffa* var Sabdariffa failed as only 31 successful hybrids (20%) out of a possible 155 were obtained (Table 4). Additionally, only two cross combinations in this group (A1 × A11 and A10 × A11) were successful in both direct and reciprocal directions. Accessions A11 and A3 emerged the most prolific female and male parents, yielding 11 and 6 successful hybrids respectively. On the other hand, A2, A13, and A14 failed to produce any hybrids in all crosses in which they were deployed as female parents.

Also, crossability estimates recorded for members of this crossing group were relatively low and varied significantly (0.00 to 43.33%). Accessions A11 and A3 recorded the highest average crossability estimates (23.00% and 13.00%) as female and male parents respectively.

Table 4. Intra-varietal crossability estimates among 14 accessions of *Hibiscus sabdariffa* var Sabdariffa

Male parent (♂)	**Female parent (♀)**														
Accession	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**A11**	**A12**	**A13**	**A14**	**Mean**
A1		0.00	0.00	0.00	0.00	*	0.00	0.00	0.00	0.00	3.33	0.00	*	*	0.00
A2	*		*	0.00	0.00	0.00	*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A3	0.00	0.00		*	0.00	*	32.33	0.00	*	0.00	0.00	0.00	0.00	16.67	2.08
A4	16.67	3.33	10.00		0.00	0.00	0.00	0.00	0.00	*	16.67	19.35	0.00	*	5.50
A5	0.00	*	23.33	*		0.00	*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.30
A6	0.00	0.00	0.00	0.00	*		0.00	0.00	0.00	*	23.33	0.00	*	0.00	2.30
A7	0.00	0.00	0.00	0.00	0.00	0.00		*	0.00	0.00	0.00	16.67	0.00	*	1.50
A8	0.00	0.00	*	0.00	23.33	0.00	0.00		0.00	0.00	*	0.00	0.00	0.00	2.60
A9	36.67	0.00	30.00	0.00	40.00	0.00	30.00	*		0.00	*	0.00	0.00	0.00	12.42
A10	0.00	0.00	20.00	0.00	0.00	0.00	13.33	*	*		26.67	0.00	*	0.00	6.00
A11	16.67	13.33	16.67	*	23.33	36.67	26.67	20.00	30.00	16.67		*	36.67	16.67	**23.00**
A12	0.00	0.00	**43.33**	0.00	0.00	*	0.00	0.00	*	*	0.00		0.00	0.00	4.30
A13	0.00	*	0.00	0.00	*	0.00	0.00	0.00	0.00	0.00	0.00	*		0.00	0.00
A14	*	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00	0.00	0.00	0.00	*		0.00
Mean	5.38	1.5	**13.03**	0.00	7.22	5.58	5.38	2.22	2.31	2.08	5.13	3.00	5.24	2.08	

Note. Bolded values represent highest values. * = Cross not carried out due to poor flowering by some parents.

3.2 Crossability Success with Respect to Time of the Day

Table 5 shows pooled data for crossability success involving 20 accessions of roselle (*Hibiscus sabdariffa*) in relation to the time of day during which crosses were made. Success rate was significantly higher during the early hours of the day but decreased steadily in subsequent hours. Crosses made at 6 am-7 am were most

successful (15.18%), followed by 7 am-8 am (10.00%), 8 am-9 am (8.94%) and 9 am-10 am (7.62%) in decreasing order.

Table 5. Variation in crossability success with respect to time of day

Time	Number of Crosses			Crossability Success (%)
	Attempted	Successful	Unsuccessful	
6 am-7 am	939	143	796	**15.18**
7 am-8 am	779	78	701	10.00
8 am-9 am	498	45	453	8.94
9 am-10 am	729	56	673	7.62
Mean	**736.25**	**80.50**	**655.75**	**10.44**

4. Discussion

4.1 Inter and Intra-Varietal Compatibility and Fertility Rates among Accessions of Hibiscus sabdariffa var Altissima and var Sabdariffa

Detailed studies on extent of crossability among potential breeding material projected as critical for achieving useful gene combinations have largely been overlooked in roselle by scientists (Baack et al., 2015). This presents an obvious challenge in the quest to speedily develop improved varieties of the crop to meet its growing demand by end-users. In this regard, the accessions used this study were selected to provide an opportunity to investigate possibility of hybridisation across and among the major mating groups of the species (*i.e. Hibiscus sabdariffa* var Altissima and *Hibiscus sabdariffa* var Sabdariffa).

The outcome of the study suggest an apparent crossability barrier between *Hibiscus sabdariffa* var Altissima and *Hibiscus sabdariffa* var Sabdariffa, implying that successful hybridisation across the two mating groups may not be achievable through conventional crossing approach. However, accessions of *Hibiscus sabdariffa* var Altissima are readily crossable with one another and also exhibit relatively high fertility which makes complete pair-wise crossing such as full diallele design (Griffing, 1956) among them easily attainable as previously reported (Louis et al., 2013).

The analyses also show that fertility rates of accessions of *Hibiscus sabdariffa* var Sabdariffa are very low, exhibiting partial incompatibility among members of this crossing group. These findings are in consonance with observations of Vaidya (2000), who also found low crossability rates during a natural hybridisation studies in *Hibiscus sabdariffa* var Sabdariffa. These outcomes denote that only partial pair-wise design (Todd, 2013) may be possible for intra-varietal crosses involving accessions of *Hibiscus sabdariffa* var Sabdariffa.

Results obtained in this study also indicate that the possibility of hybridisation largely reflects geographical proximity between accessions. This view is strengthened by the relatively high hybridisation estimates among accessions of *Hibiscus sabdariffa* var Altissima which mostly belong to the same geographical region compared to the relatively low successes recorded for *Hibiscus sabdariffa* var Sabdariffa which comprise accessions with widely differing geographical locations. Various authors have attributed such outcomes to reproductive barriers between the different ecotypes of a species (Todd, 2013; Valdiani et al., 2012).

4.2 Variation in Crossability Success with Respect to Time of Day

Percent crossability varied inversely with the course of time. This agrees with a report by (Samba, 2013) working on sweet potato (*Ipomoea batatas* L.) under the similar local conditions. It however, contradicts the report by (Li, 2000) working on kenaf (*H. cannabinus*) in China. This variation may be attributable to the different environmental conditions as the two species are closely related. Indeed, environmental conditions affect successful pollination of crops. In this study, favourable environmental conditions such as low temperature and high humidity which are usually recorded during the early hours of the day may have accounted for the comparatively higher crossability success during this period (Koike et al., 2015).

Moreover, pollen viability and stigma receptivity are known to peak in the period immediately after opening of flowers (6 am-7 am) resultant of cleistogamous mode of pollination of roselle (Vaidya, 2000). Thus, crosses made in early hours of the day are more likely to be successful than those conducted later then. Besides, increasing stigma desiccation and abscission with the course of the day may have accounting for the low success of crossability in the later part of the day (Bishop et al., 2016; Munguía-Rosas et al., 2012).

5. Conclusion

The study indicates that successful cross-fertilisation between *Hibiscus sabdariffa* var Altissima and var Sabdariffa may require special techniques beyond conventional cross pollination in the field. It was, however, observed that accessions of *Hibiscus sabdariffa* var Altissima may be crossed in a complete pair-wise design, whereas those of *Hibiscus sabdariffa* var Sabdariffa may, at best, be crossed through partial pair-wise designs. Even so, successful hybridisation among accessions of the later may be difficult to achieve. Thus, greater number of crosses should be attempted to increase the probability of successful hybridization, preferably in early hours of the day. These findings would serve as a guide to future breeding of roselle in Ghana.

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