Methyl Salicylate Affects the Quality of Hayward Kiwifruits during Storage at Low Temperature

Morteza Soleimani Aghdam (Corresponding author)

Department of Horticultural Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

Iranian Young Researchers Club, Islamic Azad University, Ahar Branch, Ahar, Iran

Tel: 98-914-759-8795   E-mail: Aghdam_1361@yahoo.com

Alireza Motallebiazar

Department of Horticultural Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

Tel: 98-914-401-9637   E-mail: Motallebiazar@gmail.com

Younes Mostofi

Department of Horticultural Science, University College of Agriculture and Natural Resources

University of Tehran, Tehran, Iran

Tel: 98-912-501-0492   E-mail: ymostofi@ut.ac.ir

Javad Fattahi Moghaddam

Iran Citrus Research Institute, Ramsar

Tel: 98-911-392-8044   E-mail: fattahi80@yahoo.com

Mahmood Ghasemnezhad

Departments of Horticultural Science, Faculty of Agriculture, University of Guilan, Rasht, Iran

Tel: 98-426-223-4857   E-mail: Ghasemnezhad@Guilan.ac.ir

Abstract

Hayward kiwifruit were treated with methyl salicylate (MeSA) at different concentrations (0, 8, 16, 24, 32 $\mu L.L^{-1}$), then stored at 0.5 ºC and 90% RH for 5 months to investigate postharvest quality and ripening behavior. MeSA treatments, especially at 32 $\mu L.L^{-1}$ concentrations, were highly effective in reducing ethylene production, fungal decay, APX and CAT activity, weight loss and total soluble solids (TSS) in Hayward kiwifruit, as well as ascorbic acid (AA) and flesh firmness loss compared with that observed in control fruit. Hayward kiwifruit treated with 24 $\mu L.L^{1}$ MeSA was lowest pH. Titrable acidity (TA) of the Hayward kiwifruit was not significantly affected by the use of MeSA treatments.

Keywords: Ascorbic acid, Ethylene, Fungal decay, Kiwifruit, Methyl salicylate

1. Introduction

Kiwifruits (*Actinidia deliciosa*) are an interesting product specially in recent years due to its high level of vitamin C and its strong antioxidant capacity due to a wide number of phytochemicals including carotenoids, phenolics, flavonoid, and chlorophyll (Cassano *et al.*, 2006). Based on these characteristics, kiwifruits offer

benefits for specific health conditions and has a great potential for industrial exploitation (Cano Pilar, 1991). Nowadays, kiwifruit is an important fruit produced in commercial scale in Iran. Kiwifruit from all growing areas may deteriorate due to infection by *Botrytis cinerea*, which causes Gray Mold decay (Snowdon, 1990). To reduce postharvest decay of fruits, it is necessary to improve plant defense mechanism and to decrease fungi contamination during plant vegetative growth, flowering, fruit development and postharvest stages.

Salicylic acid (SA) and methyl salicylate (MeSA) are endogenous signal molecules, playing pivotal roles in regulation stress responses and plant development including heat production, disease resistance, seed germination, sex polarization and ethylene production (Asgharia and Aghdam, 2010; Aghdam *et al*., 2009). SA mediated hypersensitive and systemic acquired resistances (SAR) against pathogen attack proposed to be mediated through the inhibition of catalase (CAT) and Ascorbate peroxidase (APX) activity, which subsequently raises intra-cellular H_2O_2 concentration. This increased intra-cellular H_2O_2 concentration proposed to act as a second messenger in activation and expression of defense related genes (Raskin, 1992). The SA induced defense responses are probably involved in the expression of a range of defense genes, especially those encoding PR-proteins such as phenylalanine ammonia lyase (PAL), chitinase, β-1, 3-glucanase and peroxidase (POD) (Meena *et al*., 2001). In addition, SA exhibit antifungal effects on harvested fruits (Amborabe *e*t *al*., 2000). Moreover, dietary salicylates from fruit and vegetables are described as bioactive compounds with health care potential and considered as generally recognized as safe (GRAS) (Hooper and Cassidy, 2006). SA and MeSA are known as inhibitors of ethylene biosynthesis (Leslie and Romani, 1986) and reported to inhibit wound induced transcription of ACC synthase expression and activity in tomato fruit (Li *et al*., 1992). SA can delay the ripening of banana fruit, probably through inhibition of ethylene biosynthesis or action (Srivastava and Dwivedi, 2000). Fan *et al* (1996) demonstrated the inhibitory action of SA on ACC Oxidase activity in apple fruit disks. Babalar *et al* (2007) reported that 2 mmol l^{-1} SA significantly reduced ethylene production and fungal decay and retained overall quality of Selva strawberry fruit. Treatment of banana seedling with 0.5 mM SA reduced CAT and APX activity and enhanced chilling tolerance (Kang *et al*., 2003). Chan and Tian (2006) reported that SA treatment significantly inhibited CAT activity in sweet cherry fruit. Wang *et al* (2006) reported that treatment with SA was effective in alleviating chilling injury of peach fruit. This author suggested that the effect of SA on alleviating chilling injury of peaches during cold storage may be attributed to its ability to induce antioxidant systems and heat shock proteins (HSPs). This study was carried out to investigate the effects of several MeSA concentrations on fungal decay in relation with CAT and APX activity, quality attributes such as ascorbic acid (AA) content, flesh firmness, total soluble solids (TSS), titrable acidity (TA), pH, weight loss and ethylene production in kiwifruit during postharvest storage.

2. Material and methods

2.1 Sample preparation

Uniform size fruit of kiwifruit (*Actinidia deliciosa* cv. Hayward) harvested at commercial maturity from an orchard in Citrus Research Institute in Ramsar-Iran and transferred to the laboratory on the same day. Fruit divided into five groups of 300s, comprising three replicates of 100. For MeSA vapor treatments, fruit were placed in 300-l air-tight containers, together with MeSA spotted onto filter paper at the final concentration of 0 (control), 8, 16, 24 and 32 μL.L^{-1}, respectively, followed by incubation for 16 h at 20 ºC. After these treatments the containers were opened, ventilated, and stored at 0.5 ºC and 90% RH. Evaluation was carried out for the periods of 5 months with 30 days intervals. Quality attributes such as ascorbic acid (AA) content, flesh firmness, total soluble solids (TSS), titrable acidity (TA), weight loss, ethylene production and antioxidant enzymes activity, CAT and APX evaluated during storage at 0.5 ºC and 90% RH.

2.2 Quality attributes analysis

Fruit ethylene production was determined following the method of Zhang *et al* (2003) with some modifications. Occurring this, five fruits were placed in a 1 L flask, capped with a rubber stopper for 1 h. Head space samples (1 ml) were collected by syringe and ethylene concentrations measurement by a GC (Shimadzu, GC-14A, Japan) apparatus. Ascorbic acid content measured by titration against 2, 6-dichloro-indophenol (AOAC, 1990) and results were expressed as mg of Ascorbic acid per 100 g of fresh weight (mg/100 g FW). Flesh firmness measured using a fruit pressure tester FT-011 (Facchini, Italy) on five individual fruit at each replicate by measuring force required for an 8 mm probe to penetrate in two opposite locations the mesocarp tissue. Titrable acidity was measured using titration method. To do that, 5 mL fruit juice was added to 25 mL distilled water plus two drops of phenolphthalein and titrated with 0.1N NaOH up to pH 8.1. The results were expressed as g of citric acid per 100 g fresh weight. TSS was determined using ATAGO-ATC-20E (Japan) refractometer at 20 ºC and expressed as ºBrix. The pH of fruit juice was measured using a Jenway 3320 pH meter. In order to determine

any weight loss during the storage of the fruit, both treated and untreated fruits were weighted 1, 2, 3, 4 and 5 month after treatments.

2.3 Fruit fungal decay (DI)

Fruit decay was assessed at the end of storage life, by using 30 fruit per replicate. According to the amount of the fungal mold on fruit surface scales from 1 to 5 were given to the each treatment group where; 1 = normal (no decay on fruit surface), 2 = trace (up to 5% fruit surface were decayed), 3 = slight (5–20% of fruit surface were decayed), 4 = moderate (20–50% fruit surface were decayed), and 5 = severe (>50% of fruit surface were decayed). From this, a decay index (DI) was expressed as: DI index $= \sum$ [(DI level) $\times$ (number of fruit at the DI level)] / (4×total number of fruit in the treatment).

2.4 Ascorbate peroxidase (APX) and Catalase (CAT) activity

APX activity assayed according to the method of Jimenez et al., (1997). Approximately 5 g of frozen fruit were ground with 16 ml of extraction buffer (100 mM sodium phosphate pH 7.0; 0.1 (v/v) Triton X-100; 1 M NaCl; 10 g l^{-1} PVPP) using an omnimixer. The suspension was stirred for 1 h and then centrifuged at 10,000 $\times$ g for 10 min. The resulted supernatant later was used for assaying the enzyme activity under 0-4 ºC condition. The activity was assayed at 30 ºC in a mixture containing 100 mM sodium phosphate buffer pH 7.0; 2 mM ascorbic acid; 4 mM H_2O_2 and 500 µl of enzymatic extract in a final volume of 3.0 ml. The reduction in absorbance at 290 nm was measured. One unit of enzyme activity was defined as the amount of enzyme that can oxidize 1 µmol of ascorbate at 25 ºC for 1 min. CAT activity was assayed according to the method of Chance and Maehly (1954). Approximately 5 g of frozen fruit were ground with 0.1 g PVPP and followed by addition of 10 ml of extraction buffer (74 mg DTT; 1.2 g PEG 4000 and 2.4 ml EDTA 10 mM in 240 ml phosphate buffer 0.1 M, pH 7.8). The suspension later was stirred for 1 h and then centrifuged at 20,000 $\times$ g for 15 min. The resulted supernatant was used for assaying the enzyme activity under 0-4 ºC condition. The activity was assayed in a mixture containing 31.8 µl H_2O_2 30% in 10 ml of phosphate buffer 0.1 M pH 7 (5.44 g KH_2PO_4 in 400ml H_2O mixing with 10.46 g K_2HPO_4 in 600 ml H_2O unit pH 7) and 10 µl of enzymatic extract. The reduction of absorbance at 240 nm was measured. One unit of enzyme activity was defined as the amount of the enzyme catalyzing the decomposition of 1 µmol H_2O_2 per min at 30 ºC.

2.5 Statistical analysis

Data from the analytical determinations were subjected to analysis of variance (ANOVA). Mean comparisons were performed using Duncan's test ($P < 0.05$). All analyses were performed with SPSS software package.

3. Results and Discussion

3.1 Quality attributes

As shown in Table. 1, ethylene production of the kiwifruit was significantly decreased by treating with MeSA (P < 0.01). Control fruit had the highest rate of ethylene production at all evaluation times, while the lowest rate of ethylene production occurred in 32 µL.L^{-1} treatment. In other hand, ethylene production in fruit decreased with increasing of MeSA concentration and the most effective MeSA concentration was 32 µL.L^{-1}. Ethylene plays a key role in fruit ripening and senescence. This hormone triggers the induction of cell wall hydrolyzing enzymes leading to increase in respiration rate, fruit softening and senescence (Wills et al., 1998). In this study, MeSA treatment significantly decreased ethylene production in Hayward kiwifruits. Both SA and ASA have been shown to inhibit ethylene production in cultured pear cells, mung bean hypocotyls, apple and pear fruit tissue discs, carrot cell suspension cultures and strawberry fruit (Babalar et al., 2007; Romani et al., 1989). Srivastava and Dwivedi (2000) reported that SA has delayed the ripening of banana fruit, probably through inhibition of ethylene biosynthesis or action. SA decreases ethylene production by decreasing ACS and ACO production and activity (Asgharia and Aghdam, 2010). Zhang et al (2003) reported that postharvest treatment of kiwifruit with acetyl salicylic acid (ASA) resulted in a lower ACO and ACS activity and decreased ethylene production during the early stages of fruit ripening. MeSA treatment maintained firmness of fruit flesh significantly (P<0.01; Table 2) during storage. There was a positive correlation between MeSA concentration and fruit firmness. The highest fruit firmness was observed when 32 µL.L^{-1} MeSA applied at all determination times, while the lowest rate of firmness was related to control fruits. Softening of fruits is a main and critical quality changes during storage. In this study, MeSA, in a concentration dependent manner from 0 to 32 µl L^{-1}, maintained firmness of kiwifruit during storage. Srivastava and Dwivedi (2000) reported that when bananas treated with SA, fruit softening markedly decreased. Zhang et al (2003) reported a positive correlation between fruit free SA content and firmness in kiwifruit during ripening. It has been demonstrated that SA decreased ethylene production and

inhibited cell wall and membrane degrading enzymes leading to decreasing the fruit softening rate (Srivastava and Dwivedi, 2000; Zhang *et al.*, 2003).

As shown in Table 3, TSS increased during postharvest storage and MeSA treatment significantly affected TSS ($P< 0.05$). The most effective MeSA concentration was 32 µL.L^{-1} and of 14.8 % in control fruit received to 13.5 % in fruits treated with 32 µL.L^{-1}. As seen in Table 3, pH of fruit juice increased after 3 month of storage beginning but then decreased to end of storage. MeSA treatment significantly affected pH ($P < 0.05$). pH of fruits who treated with 24 µL.L^{-1} was 3.52, while in control fruit was 3.40. Generally, pH of control fruits increased during postharvest storage, but those treated by MeSA were decreased. Titrable acidity was not significantly affected by using of MeSA treatments. TSS and soluble sugars may increase during fruit ripening due to the action of sucrose-phosphate synthase (SPS), a key enzyme in sucrose biosynthesis (Hubbard *et al.*, 1991). This enzyme is activated by the ripening process, ethylene, and cool storage (Langenkämper *et al.*, 1998). Recently, an increase in SPS and invertase activities and a decrease in sucrose synthase activity have been reported during ripening of some fruits (Cordenunsi and Lajolo, 1995). In this study treatment of kiwifruits with MeSA (32 µL.L^{-1}) maintained a lower content of TSS than the control fruits at the end of cold storage. We propose that MeSA reduced ethylene production may results to decreased SPS enzyme activity leading to decrease in sucrose synthesis and TSS content. Cell walls contain large amounts of polysaccharides, mainly pectins and cellulose, and are digested due to the activity of the cell wall degrading enzymes leading to a significant increase in TSS content. SA effectively protects cell walls by decreasing the expression of degrading enzymes and as a consequence prevents from dramatic increase in TSS content of the cells (Asghari and Aghdam, 2010).

Weight loss of the kiwifruit was significantly decreased when they were treated by MeSA ($P < 0.01$; Table 2). Control fruits had the highest of weight loss at all determination times, while the lowest of weight loss occurred in 32 µL.L^{-1} MeSA concentration. In additional, weight loss in fruit decreased with increasing of MeSA concentration. It has been demonstrated that SA in a concentration dependent manner effectively reduces respiration in plants and harvested fruits (Srivastava and Dwivedi, 2000; Han *et al.*, 2003; Wolucka *et al.*, 2005). Decrease in fruit metabolic activities results to decrease in fruit water content, weight loss, carbohydrate depletion rate and consequently, effectively delays fruit senescence process (Wills *et al.*, 1998). In this study kiwifruit treated with 32 µL.L^{-1} MeSA had the lowest weight loss during postharvest storage and was negative correlation between concentration of MeSA and weight loss in storage. During storage, MeSA treatment maintained significantly (P < 0.01; Table. 2) ascorbic acid content of the fruit being a positive correlation between MeSA concentration and fruit ascorbic acid content. The highest ascorbic acid content was observed in fruit treated with 32 µL.L^{-1} at all determination times, while the lowest ascorbic acid content was related to control fruits. Ascorbate (AA), as an essential metabolite and powerful regulator of cell functions, play a critical role in antioxidant defense system (Smimoff, 1995). In our work, AA content in MeSA treated fruit were higher than that control fruits. Hung *et al* (2007) suggested that high AA contents in the pulp of pretreated fruit with SA may result from an acceleration of biosynthetic pathways or a decrease in catabolism through an accumulation of dehydroascorbate (DHAA). Accumulation of DHAA suggests that catabolism may be an important reason for this. Both changes lead to a shift from the reduced form to the oxidized form, to a decrease in the AA/DHAA ratios. In grape plants, salicylic acid enhanced PM-Ca^{2+}-ATPase and V-Ca^{2+}-ATPase activity and induced Ca^{2+} movement from vacuoles and intercellular spaces to the cytoplasm, higher cytosolic Ca^{2+} might induce the Ascorbate-Glutathione cycle (GR activity increased and high GR activity maintains the pool of GSH, allowing GSH to be used by DHAR to reduced DHA to AA), causing GSH and AA to increase and salicylic acid pretreated plants maintaining higher AA/DHAA and GSH/GSSG rate (Wang and Li, 2006). Hung *et al* (2007) reported that activities of GR and DHAR and the content of AA and GSH in Cara cara navel orange during fruit storage declined but the SA-pretreatment reduced the rate of this decline and the SA-pretreated fruit had higher values of AA/DHAA and GSH/GSSG than those in controls.

3.2 Fruit fungal decay (DI), CAT and APX activity

DI was significantly (P < 0.01; Table. 3) affected by MeSA vapor in the end of shelf life period so that DI in the fruits treated with 32 µL.L^{-1} MeSA were 6.3% whereas it was 34.2% in control fruits. CAT and APX activity gradually increased during storage while their activity were significantly (P < 0.01; Table 4) reduced in MeSA treated fruit. The lowest CAT and APX activity observed when 32 µL.L^{-1} MeSA applied at all determination times, while the highest CAT and APX activity was related to control fruits. In totally, CAT and APX activity in fruit decreased with MeSA concentration increased especially in 32 µL.L^{-1} MeSA concentration. Exogenous application of SA at nontoxic concentrations to susceptible fruits and vegetables could enhance resistance to pathogens and control postharvest decay (Asgharia and Aghdam, 2010; Aghdam *et al.*, 2009). SA in a

concentration dependent manner from 1 to 2 mmol $L^{-}1$ effectively reduced fungal decay in Selva strawberry fruit (Babalar *et al.*, 2007). MeSA triggers disease resistance and mediates the expression of defense related genes in neighboring plants and in healthy tissue of infected plants (Shulaev *et al.*, 1997). Hayward kiwifruit postharvest decay was significantly affected by MeSA vapor at the end of storage period. Decay incidence in fruit treated with 32 $\mu L.L^{-1}$ was 6.3% whereas it was 34.2% in control fruits. Dipping of pear fruit in 1 mmol L^{-1} SA solution effectively controlled fruit decay during 5 months of cold storage (Asghari *et al.*, 2007). Postharvest treatment of table grapes with SA before coating with chitosan significantly enhanced the efficiency of coating and decreased fruit decay (Asghari *et al.*, 2009). SA also exhibits direct antifungal effects against pathogens. 2 mmol L^{-1} SA showed direct fungal toxicity on *Monilinia fructicola* and significantly inhibited the mycelia growth and spore germination of the pathogen in *in vitro* (Yao and Tian, 2005). According to the results of Qin *et al* (2003), 0.5 mmol L^{-1} SA significantly reduced the incidence of blue mould (*P. expansum*) and alternaria rot (*A. alternata*) in sweet cherry without any surface injury. Adding SA significantly improved the activity of *R. glutinis* against both pathogens. Qin *et al* (2003) demonstrated that SA treatment can stimulate the synthesis of antioxidant enzymes in sweet cherry fruit and induces a significant increase in the activities of polyphenoloxidase (PPO), phenylalanine ammonia lyase (PAL) and β-1, 3-glucanase. Zeng *et al* (2006) suggested that PAL and *β*-1, 3-glucanase, as well as H_2O_2 or $O_2^{\cdot-}$, may be involved in the enhancement of disease resistance in mangoes. Kiwifruit treatment with MeSA resulted in a significant decrease in fungal decay confirming the fact that MeSA leads to the activation of plant defense system against pathogens. Pretreatment of kiwifruit with MeSA reduce CAT and APX activity and negative correlation exist between MeSA concentration and CAT and APX activities. The lowest CAT and APX activity was observed when 32 $\mu L.L^{-1}$ MeSA applied at all determination times, while the highest activities of CAT and APX were related to controls.

4. Conclusions

In conclusion, the role of MeSA in reducing postharvest decay in Hayward kiwifruit has been addressed. From the assayed concentrations, 32 $\mu L.L^{-1}$ as the most effective for reducing CAT and APX activity and DI, ethylene production, weight loss, TSS and for maintenance of AA levels and flesh firmness. However, more in-depth experiments should be carried out to understand the mechanism by witch MeSA improves the postharvest quality of Hayward kiwifruit.

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Table 1. Effects of different combinations of MeSA concentrations and time of analysis on Hayward kiwifruit ethylene production (nL/Kg/h)

Quality attribute	Treatment	Storage time (month)			
		1	2	3	4
Ethylene	Control	80.33p	126.66k	138.66g	182e
(nL/Kg/h)	MeSA 8 µL.L	71.66q	102.33l	126.66i	164.33g
	MeSA 16µL.L	60.66r	90.66m	112.66j	151.66h
	MeSA 24µL.L	50.66s	79.66n	100k	145.33i
	MeSA32µL.L[1]	42.66t	53o	87.66n	132.33j

Means with the same letter in a column are not significantly different at *P*< 0.01 (Duncan's test).

Table 2. Effects of different combinations of MeSA concentrations and time of analysis on Hayward kiwifruit fruit firmness (N), vitamin C (mgr/100gr FW) content and weight loss (gr)

Quality attribute	Treatment	Storage time (month)				
		1	2	3	4	5
Firmness	Control	5.5e	3.5k	2.4mno	1.8q	1.03l
(N)	MeSA 8 µL.L^{-1}	5.9d	3.9hi	2.6m	1.8q	1.2s
	MeSA 16 µL.L^{-1}	6.2c	4.2g	3.1l	2.2p	1.5r
	MeSA 24 µL.L^{-1}	6.5b	4.9f	3.8i	2.5m	2.4mn
	MeSA 32 µL.L^{-1}	7a	5.5e	4.3g	3.2l	2.2o
Vitamin C	Control	60g	55jk	49.3m	44.6n	41o
(mgr/100gr FW)	MeSA 8 µL.L^{-1}	63e	60.3fg	56ij	51l	46n
	MeSA 16 µL.L^{-1}	67d	62.6e	60.3fg	55jk	50lm
	MeSA 24 µL.L^{-1}	71b	65.6d	61.6ef	56.6hi	53.6k
	MeSA 32 µL.L^{-1}	75a	69c	65.6d	61fg	58h
Weigh loss	Control	1.5n	2k	2.8h	3.8e	6.7a
(g)	MeSA 8 µL.L^{-1}	1.4o	1.8l	2.5i	3.5f	5.3b
	MeSA 16 µL.L^{-1}	1.2p	1.5mn	2.1jk	2.9g	4.6c
	MeSA 24 µL.L^{-1}	1.1q	1.4o	1.8l	2.6i	4d
	MeSA 32 µL.L^{-1}	1r	1.3p	1.6m	2.1j	3.5f

Means with the same letter in a column are not significantly different at *P*< 0.01 (Duncan's test).

Table 3. Effects of MeSA concentration on Hayward kiwifruit TSS, pH, DI % and TSS/TA

Quality attribute	Treatment (MeSA µL.L[1])				
	Control	8	16	24	32
TSS	14.7b	14.39b	14.39b	13.84a	13.70a
pH	3.52bc	3.48b	3.45ab	3.40a	3.45ab
DI%	34.3e	28d	20.6c	13.3b	6.3a
TSS/TA	20.06b	19.84b	19.67b	17.89a	19.53b

Means with the same letter in a column are not significantly different at $P< 0.05$ (Duncan's test).

Table 4. Effects of different combinations of MeSA concentrations and time of analysis on CAT and APX activity in Hayward kiwifruit

Quality attribute	Treatment	Storage time (month)		
		1	3	5
APX activity	Control	0.035f	0.044c	0.057a
	MeSA 8 µL.L^{-1}	0.03h	0.038c	0.050b
	MeSA 16 µL.L^{-1}	0.025i	0.029h	0.041d
	MeSA 24 µL.L^{-1}	0.02j	0.025i	0.032g
	MeSA 32 µL.L^{-1}	0.015k	0.018j	0.025i
CAT activity	Control	0.076ed	0.090c	0.12a
	MeSA 8 µL.L^{-1}	0.065f	0.079d	0.10b
	MeSA 16 µL.L^{-1}	0.054g	0.065f	0.08c
	MeSA 24 µL.L^{-1}	0.040h	0.055g	0.073e
	MeSA 32 µL.L^{-1}	0.033i	0.043h	0.061f

Means with the same letter in a column are not significantly different at $P< 0.01$ (Duncan's test).