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Foliar Application of Mixture of Amino Acids and Seaweed (*Ascophylum nodosum*) Extract Improve Growth and Physico-chemical Properties of Grapes

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ABSTRACT

Foliar applications of mixture of amino acids and *Ascophylum nodosum* (Seaweed) extract at different growth stages were investigated on the growth and physico-chemical characteristics of grapes cv. 'Perlette'. Grapevines were sprayed with 0.5 mL L^{-1} aqueous solution of mixture of amino acids and seaweed extract plus 0.01% Tween-20 as surfactant at flowering stage, fruit setting stage, flowering + fruit setting stages or flowering + fruit setting + one month after fruit setting stages. Unsprayed grapevines were kept as control. Grapevines treated with multiple applications of mixture of amino acids and seaweed extract at flowering + fruit setting + one month after fruit setting stages exhibited significantly higher leaf size (41.5%), chlorophyll content (18.15 mg g^{-1}), berry setting (6.66%), number of bunches per cane (6.66%), rachis length (13.5%), berry weight (14.78%), berry size (7.33%), soluble solid concentrations (SSC, 16%), SSC: titrable acidity (TA) ratio (29), pH of juice (3%), total sugars (28%) and reducing sugars (35%) with reduced berry drop (10.6%) and ascorbic acid (28.6%) contents as compared to all other treatments. Treated grapevines showed no significant change in the leaf mineral contents, bunch size and weight. In conclusion, multiple foliar applications of 0.5 mL L^{-1} mixture of amino acids and seaweed extract can be used effectively to improve growth and physico-chemical berry quality of grapevine cv. 'Perlette'.

Key Words: *Vitis vinifera*; Chlorophyll content; Seaweed extract; Perlette; Primo

INTRODUCTION

Grapevine [European (*Vitis vinifera* L.) or American (*Vitis labrusca* L.) or Muscadine (*Vitis rotundifolia* L.)] is one of the earliest fruit crop grown in the world (Salunkhe & Kadam, 1995). It occupies top position in the world with respect of area (75866 thousand ha) and production (67221 thousand tones) (FAOSTAT data, 2010). However, in Pakistan it is ranked as 10th important fruit crop (GOP, 2010). In the year 2008-2009, the grape production in Pakistan was estimated to be 122 thousand tones on an area of about 14 thousand ha (GOP, 2010). This was the highest production in the last nine years. Presently, in Pakistan over 70% of grapes are being produced in Balochistan with 19 tons ha^{-1} yield against the potential of 25 tons ha^{-1} (Khan *et al*., 2011).

Long, warm and dry summer with cool winter is considered best for production of grapes with higher yield and superior berry quality. Mountainous and sub-mountainous areas up to 2,000 m or even more elevation are considered best for its cultivation (Winkler *et al*., 1974). However, early maturing cultivars can be grown in central Punjab as monsoon rains at the time of berry ripening lead to development of fungal diseases and rapid berry rottening. 'Perlette' being an early maturing cultivar of grapes can be successfully grown on commercial scale in Punjab, Pakistan. However, occurrence of shorter berries with small rachis length leads to development of bunch compactness which consequently reduces its market value. Earlier various management techniques such as exogenous application of low biuret urea and different levels of pruning have been used to overcome these problems but the results are sporadic and inconclusive (Ahmed *et al*., 2004a & b).

Seaweed extract being organic and biodegradable in nature is considered as an important source of nutrition for sustainable agriculture (Cassan *et al*., 1992). Seaweeds contain various trace elements (Fe, Cu, Zn, Co, Mo, Mn & Ni), vitamins, amino acids and plant growth hormones (IAA, IBA & Cytokinins) which cause many beneficial effects on plant growth and development (Metting *et al*., 1990; Spinelli *et al*., 2009; Abdel-Mawgoud *et al*., 2010). The extract of seaweeds has been reported to induce many positive changes in treated plants such as improved crop yield, increased nutrient uptake, resistance to frost and stress conditions, increased postharvest shelf life, increased seed germination and reduced incidence of fungal and insect

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attack (Metting *et al*., 1990). Foliar applications of seaweed extract has been reported to influence growth, productivity and fruit quality of some fruit crops including 'Red Roomy' (Abada, 2002), 'Superior' (Abd El-Wahab, 2007; Abd El Moniem & Abd-Allah, 2008) and 'Thompson Seedless' (Abd El-Ghany *et al*., 2001) grapes, 'Keitte' mango (Abd El-Motty *et al*., 2010), water melon (Abdel-Mawgoud *et al*., 2010) and apple (Spinelli *et al*., 2009) fruits with variable effects.

Amino acids are considered as precursors and constituents of proteins (Rai, 2002), which are important for stimulation of cell growth. They contain both acid and basic groups and act as buffers, which help to maintain favorable pH value within the plant cell (Davies, 1982). Amino acids can directly or indirectly influence the physiological activities in plant growth and development such as exogenous application of amino acids have been reported to modulate the growth, yield and biochemical quality of squashes and garlic plants (El-Shabasi *et al*., 2005; Abd El-Aal *et al*., 2010; Shiraishi *et al*., 2010). However, effects of foliar application of amino acids on the growth and quality of grapevine warrant investigation.

At present, very little is known about the effects of foliar application of amino acids and seaweed extract on the growth, productivity and berry quality of grapes. Whereas, to the best of our knowledge this is the first study in which combine foliar application of amino acids and seaweed extract has been investigated on the growth and quality of grapes. We hypothesized that the exogenous application of mixture of amino acids and seaweeds will improve the growth and berry quality in grapes. Hence, a preliminary study was carried out to test the efficacy of a commercially available product 'Primo' (a mixture of amino acids & seaweed extract) on grapevine growth and berry quality of cv. 'Perlette' grown under agro-climatic conditions of Faisalabad.

MATERIALS AND METHODS

Plant materials: The present study was carried out during 2010 in the Experimental Fruit Garden, Institute of Horticultural Sciences (31°25'N; 73°09'E), University of Agriculture, Faisalabad, Pakistan. Thirty years old grape vines (*Vitis vinifera* L.) cv. 'Perlette' having uniform vigor and health, trained on pergola training system with 15 × 15 ft plant and row distance were selected for the experimental purpose. All the grapevines received uniform management practices including pruning (up to six nodes), irrigation, fertilizer, insecticide and pesticide applications. The grapevines were sprayed with aqueous solutions of 0.5 mL L^{-1} 'Primo' [a mixture of amino acids (20%) and seaweed extract (12%)] + 0.01% Tween-20 as surfactant at flowering stage (T2, April 5, 2010), fruit setting stage (T3, April 14, 2010), flowering + fruit setting stages (T4), or flowering + fruit setting + one month after fruit setting stages (T5, May 11, 2010). All the grapevines were thoroughly sprayed till run off using hand held knapsack sprayer. Unsprayed grapevines were kept as control (T1). Two grapevines were taken as experimental unit to record the data with three replicates. Data for vine characteristics was recorded in the field, while leaves and fruit samples were taken to the Pomology Lab., Institute of Horticultural Sciences, University of Agriculture, Faisalabad, for further analysis.

Leaf mineral contents: Leaf nitrogen (N) contents were determined by using micro Kjeldhal, phosphorus (P) by Vanadomolybdo, and potassium (K) by flame photometer methods, respectively as described by Chapman and Parker (1961). For determination of leaf manganese (Mn) and iron (Fe) contents, wet digestion was done as outlined by (Yoshida *et al*., 1976) and were estimated by atomic absorption spectrophotometer (2-8200 Series, Polarized Zeeman, Hitachi, Japan) using specific lamp for specific nutrient. The boron (B) contents in leaf samples were determined by dry ashing method (Chapman & Pratt, 1978) and further measurement of B were taken by colorimetry using Azomethine-H (Bingham, 1982).

Vegetative and reproductive growth: For study of vegetative and reproductive behavior five canes were tagged on each experimental grapevine. Five fully expanded leaves per cane were collected and their leaf sizes were measured by using digital leaf area meter and were expressed as cm^2. Leaf chlorophyll meter (SPAD-502 Meter, Minolta, Japan) was used to determine the leaf chlorophyll contents of experimental grapevines and was expressed as SPAD value. Total numbers of flowers per bunch, number of berry setting per bunch and berry drop per bunch were counted on each flowers cluster on the tagged cane and berry set and berry drop was calculated as percentage. Data regarding berry size (cm^2), hundred berry weight (g), numbers of berries per bunch, numbers of punches per cane, rachis length (cm), bunch weight (g), bunch size (cm^2) were also collected by using standard procedures (Abu-Zahra, 2010; Khan *et al*., 2011).

Berry physico-chemical characteristics: A digital refractometer ATAGO, RS-5000 (Atago, Japan) was used to determine soluble solid contents (SSC) of berry juice. For pH determination, 20 mL of berry juice was taken in a beaker and pH was recorded using digital pH meter (HI 98107, Hanna Instruments, Mauritius). Titretable acidity (TA) of berry juice was determined by method described by Khan *et al*. (2011). Ascorbic acid contents and sugars in the berry juice were determined the method outlined earlier by Khan *et al*. (2011).

Statistical analysis: The data were subjected to one-way analysis of variance (ANOVA) under Randomized Complete Block Design using Genstat (release 31.1; Lawes Agricultural Trust, Rothamsted Experimental Station, Rothamsted, UK). Two grapevines were used as experimental unit replicated three times. The effects of various treatments were assessed within ANOVA and Fisher's least significant differences were calculated following a significant ($P \leq 0.05$) F test. All the assumptions of analysis were checked to ensure validity of statistical analysis.

RESULTS AND DISCUSSION

Foliar application of mixture of amino acids and seaweed extract did not significantly influence the mineral contents of grapevine leaves (Table I). However, grapevines treated with multiple spray applications of mixture of amino acids and seaweed extract at flowering + fruit setting stages showed higher levels of leaf N, P, K, B, Fe and Zn contents as compared to all other treatments. Similarly, Norrie *et al*. (2002) also reported that foliar application of *Ascophylum nodosum* extract had no effect on mineral contents of grapevine leaves. Grapevines subjected to multiple spray applications of mixture of amino acids and seaweed extract at flowering + fruit setting + one month after fruit setting stages exhibited highest increase in leaf size about 1.7-fold higher than untreated grapevines (Fig. 1a). Increase in leaf size may be ascribed to the hormonal action of seaweed extract, which increased the endogenous hormonal level of treated grapevines. *A. nodosum* extract has been found to contain biologically active cytokinins, which promote cell division (Miller, 1961). Recently Abdel-Mawgoud *et al*. (2010) observed that foliar application of seaweed extract significantly increased size of water melon leaves.

Foliar application of 0.5 mL L^{-1} mixture of amino acids and seaweed extract at different growth stages significantly improved leaf chlorophyll contents in grapevines (Fig. 1b), however, grapevines treated with multiple spray applications at flowering + fruit setting + one month after fruit setting stages showed highest 19% increase in leaf chlorophyll contents than untreated grapevines. This increase in chlorophyll contents is might be due to availability of higher levels of amino acids to the treated plants (El-Shabasi *et al*., 2005; Awad *et al*., 2007). Similarly, exogenous application of seaweed extract (Actiwave) resulted in 12% increase in the chlorophyll contents in 'Fuji' apple leaves with a consequent increase in the photosynthesis and respiration rates (Spinelli *et al*., 2009).

Grapevines sprayed with mixture of amino acids and seaweed extract exhibited significant ($P \leq 0.5$) increase in the berry setting than control (Fig. 2a). The highest increase in berry setting (7%) was recorded in the grapevines subjected to the multiple applications of 0.5 mL L^{-1} mixture of amino acids and seaweed extract at flowering + fruit setting + one month after fruit stages than control. Similar to berry setting, the grapevines treated with multiple applications of 0.5 mL L^{-1} mixture of amino acids and seaweed extract at flowering + fruit setting + one month after fruit stages exhibited 10.66% reduction in the berry drop than control (Fig. 2b). The increase in berry setting and reduction in berry drop may be due to the positive effect of seaweed extract on endogenous levels of growth promoters, amino acids and carbohydrates (Kulk, 1995). Earlier spraying of 2% algae + 0.2% yeast extract at full bloom had been reported to increase the fruit setting and fruit retention percentages in 'Kiette' mango (Abd El-Motty *et al*., 2010).

Fig. 1: Effect of foliar application of mixture of amino acids and seaweed extract (0.5 mL L^{-1}) at different growth stages on leaf size and chlorophyll contents of grapes cv. Perlette. Vertical bars indicate ± SE of means. n = 3 replicate

Treatment details for all figures: T1 = Control. T2 = at flowering stage. T3 = at fruit setting stage. T4 = at flowering and fruit setting stages. T5 = at flowering, fruit setting and one month after fruit setting stages

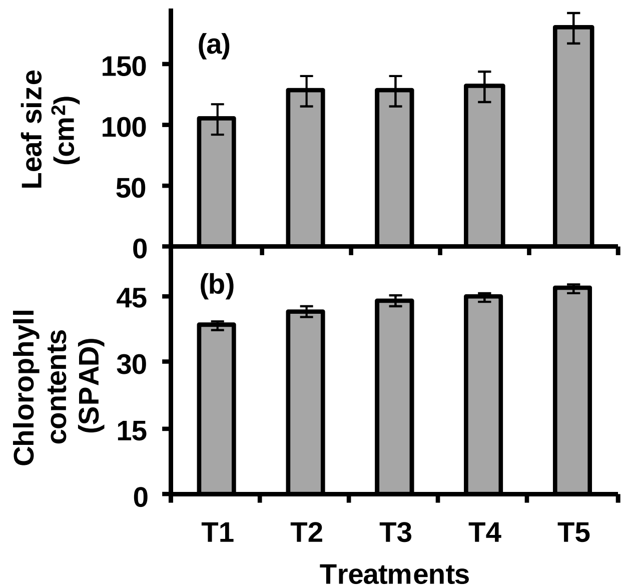


Fig. 2: Effect of foliar application of mixture of amino acids and seaweed extract (0.5 mL L^{-1}) at different growth stages on berry setting and berry drop of grapes cv. Perlette. Vertical bars indicate ± SE of means. n = 3 replicate

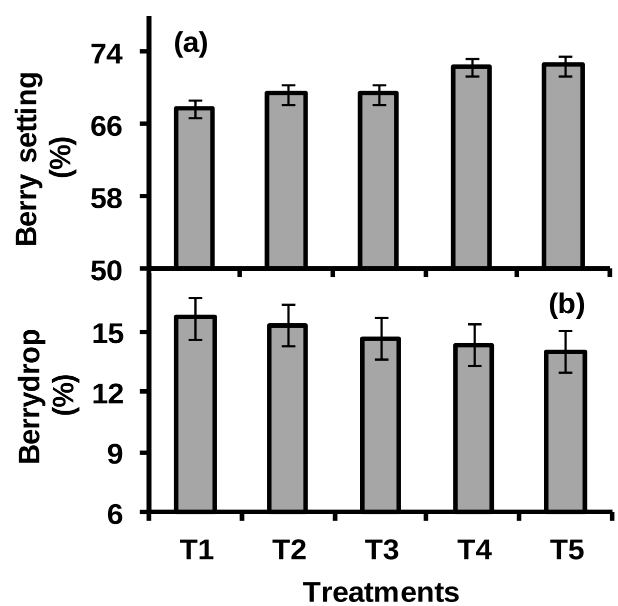


Table I: Effect of foliar application of mixture of amino acids and seaweed extract at different growth stages on leaf mineral contents of grapes cv. Perlette

Foliar spray (0.5 mL L^{-1})	N (%)	P (%)	K (%)	B (mg/kg)	Fe (mg/kg)	Mn (mg/kg)
Control (no spray)	1.92 ± 0.21	0.47 ± 0.01	0.67 ± 0.03	25.33 ± 2.1	94.0 ± 2.5	100.0 ± 5.6
At flowering	1.74 ± 0.35	0.46 ± 0.01	0.64 ± 0.02	25.66 ± 1.9	94.0 ± 3.4	100.7 ± 6.9
At fruit setting	1.86 ± 0.21	0.45 ± 0.02	0.64 ± 0.03	25.33 ± 1.1	94.3 ± 3.2	101.0 ± 7.2
At flowering + fruit setting	1.98 ± 0.20	0.58 ± 0.01	0.69 ± 0.04	25.86 ± 1.2	96.7 ± 4.2	101.7 ± 6.3
At flowering + fruit setting + month after fruit setting	1.86 ± 0.20	0.53 ± 0.02	0.66 ± 0.02	26.0 ± 1.5	95.0 ± 2.1	100.7 ± 5.4
LSD ($P \leq 0.05$)	NS	NS	NS	NS	NS	NS

Any two means ± SD in a column followed by same letters are not significant at ($P \leq 0.05$). NS = not significant; n = 3

Table II: Effect of foliar application of mixture of amino acids and seaweed extract at different growth stages on reproductive growth of grapes cv. Perlette

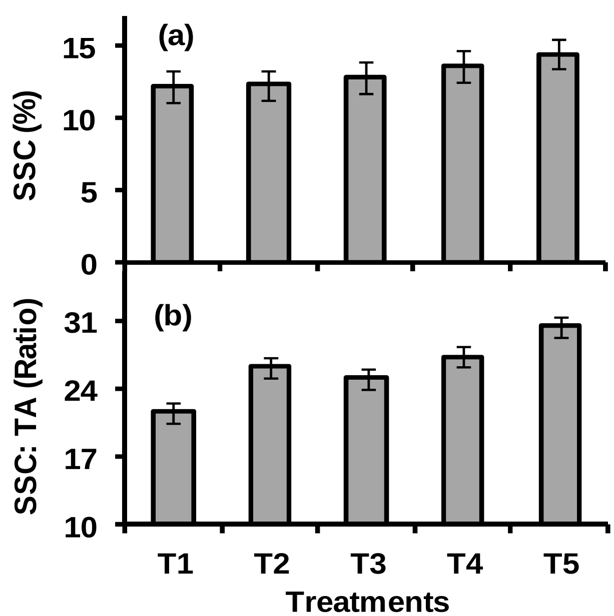
Treatments (0.5 mL L^{-1})	No. of bunches per cane	Rachis length (cm)	Bunch size (cm^2)	Bunch weight (g)	No. of berries per bunch	Berry size (cm^2)	100 berry weight (g)
Control (no spray)	2.6 ± 0.3c	15.45 ± 1.2b	146.7 ± 10.8	435.3 ± 52	438 ± 41e	0.47 ± 0.02b	98.5 ± 7.2b
At flowering	2.6 ± 0.3c	15.50 ± 0.3b	145.4 ± 16.1	443.0 ± 35	448 ± 39d	0.48 ± 0.01ab	98.9 ± 8.1b
At fruit setting	3.0 ± 0.2bc	15.53 ± 0.5b	143.6 ± 8.9	452.3 ± 47	457 ± 42c	0.48 ± 0.01ab	99.4 ± 9.2b
At flowering + fruit setting	3.9 ± 0.1ab	17.21 ± 1.0ab	155.7 ± 9.3	501.0 ± 42	468 ± 34b	0.50 ± 0.01a	107.8 ± 7.2ab
At flowering + fruit setting + month after fruit setting	4.2 ± 0.1a	17.94 ± 1.3a	165.8 ± 8.7	513.0 ± 51	478 ± 35a	0.50 ± 0.01a	115.5±11.1a
LSD ($P \leq 0.05$)	0.45	2.13	NS	NS	9.45	0.031	7.71

Any two means ± SD in a column followed by same letters are not significant at ($P \leq 0.05$). NS = not significant; n = 3

Data pertaining to number of bunches per cane, rachis length, numbers of berries per bunch, berry size and one hundred berry weight showed significant ($P \leq 0.5$) increase in the grapevines treated with foliar application of mixture of amino acids and seaweed extract, whereas, the foliar application of mixture of amino acids and seaweed extract did not influenced the bunch size and bunch weight than control (Table II). Multiple applications of 0.5 mL L^{-1} mixture of amino acids and seaweed extract at flowering + fruit setting + one month after fruit stages resulted in the highest 38, 14, 9, 6 and 16% increase in number of bunches per cane, rachis length, numbers of berries per bunch, berry size and one hundred berry weight than untreated grapevines, respectively (Table II). The increase in number of bunches per cane may be due to increased synthesis of photosynthates in the treated grapvines (Abd El-Aal *et al.*, 2010). The obtained results are in line with Norrie and Keathley (2006) who reported that application of seaweed extract to 'Thompson Seedless' grape increased the number of primary bunches per vine. These obtained results may be due to availability of iron and manganese contents in seaweed extract, which enhanced chlorophyll production (Fig. 1b) and photosynthesis processes which led to positive effects on growth parameters. The increase in berry weight is ascribed to the increased of chlorophyll contents of leaves, which increased photosynthesis and ultimately overall health of vine. Earlier, exogenous application of seaweed extract has been reported in 26.5% increase in 'Thomson Seedless' grapes (Norrie & Keathley, 2006). Same results were obtained by Spinelli *et al*. (2009) who described that application of seaweed extract (Actiwave) increased fruit size in apple trees.

Results showed that foliar application of mixture of amino acids and seaweed extract at different growth stages increased the SSC and SSC:TA ratio of the berry juice (Fig. 3). Maximum 1.3 and 1.5-fold increase in SSC and SSC:TA ratio of berry juice were exhibited by the berries harvested from grapevines sprayed with 0.5 mL L^{-1} mixture of amino acids and seaweed extract at flowering + fruit setting + one month after fruit stages than control. Increase in SSC and SSC:TA ratio may be related with enzymes which are present in seaweed extract that enhanced the synthesis of

Fig. 3: Effect of foliar application of mixture of amino acids and seaweed extract (0.5 mL L^{-1}) at different growth stages on SSC, TA and SSC:TA of grapes cv. Perlette. Vertical bars indicate ± SE of means. n = 3 replicate



different proteins, acids and sugars. Abd El Moniem described that foliar application of green algae extract on 'Superior' grapevines significantly improved SSC synthesis. Similarly, Abd El-Ghany *et al.* (2001) and Ismaeil *et al.* (2003) also observed that application of bio-stimulants improved the SSC:TA ratios in 'Roomy Red' and 'Thompson seedless' grapes.

Grapevines did not exhibit any significant change in the berry juice pH values with single spray application of 0.5 mL L^{-1} mixture of amino acids and seaweed extract at flowering stage or fruit setting stage as compared to its multiple applications (Fig. 4a). Foliar applications of 0.5 mL L^{-1} mixture of amino acids and seaweed extract at flowering + fruit setting stages or flowering + fruit setting + one month after fruit setting stages resulted in significant increase in the pH level of berry juice as compared to all other treatments. Smith (2008) described that exogenous application of amino acids can increase pH of treated fruit. Berries harvested from all the treated grapevines exhibited significant ($P \leq 0.5$) increase in the ascorbic acid contents in berry juice than control (Fig. 4b). Highest increase in the level of ascorbic acid was observed in the berries harvested from the grapevines subjected to multiple applications of 0.5 mL L^{-1} mixture of amino acids and seaweed extract at flowering + fruit setting + one month after fruit stages. The increase in ascorbic acid contents of treated berries may be ascribed to the combine effect of amino acids and enzymes present in seaweed extract. Similarly, Abd El-Motty *et al.* (2010) observed significant increase in ascorbic acid contents of 'Keitte' mango fruit with foliar application of 2.0% algae and 0.2% yeast extract.

The levels of reducing, non-reducing and total sugars in the berry juice significantly ($P \leq 0.5$) increased with the foliar application of mixture of amino acids and seaweed extract (Fig. 5). Highest and increase in the reducing sugars (1.3-fold) and total sugars (1.4-fold) were recorded in the berries harvested from the grapevines sprayed with mixture of amino acids and seaweed extract at flowering + fruit setting + one month after fruit setting stages (Fig. 5a & c). Grapevines sprayed with mixture of amino acids and seaweed extract at flowering + fruit setting stages exhibited 1.3-fold higher level of non-reducing sugars in the berry juice as compared to all other treatments (Fig. 5). Enhanced level of leaf chlorophyll in the treated grapevines ultimately resulted in increased rate of photosynthesis and accumulation of carbohydrate reserves in the vines. Similarly, increase in the total sugar contents have been reported in apple with foliar application of seaweed extract (Spinelli *et al*., 2009). Abada (2002) also reported that foliar application of yeast extract and some micronutrients increased the reducing sugars percentage of 'Red Roomy' grapevines.

CONCLUSION

Foliar application of mixture of amino acids and

Fig. 4: Effect of foliar application of mixture of amino acids and seaweed extract (0.5 mL L^{-1}) at different growth stages on ascorbic acid contents and pH of grapes cv. Perlette. Vertical bars indicate ± SE of means. n = 3 replicate

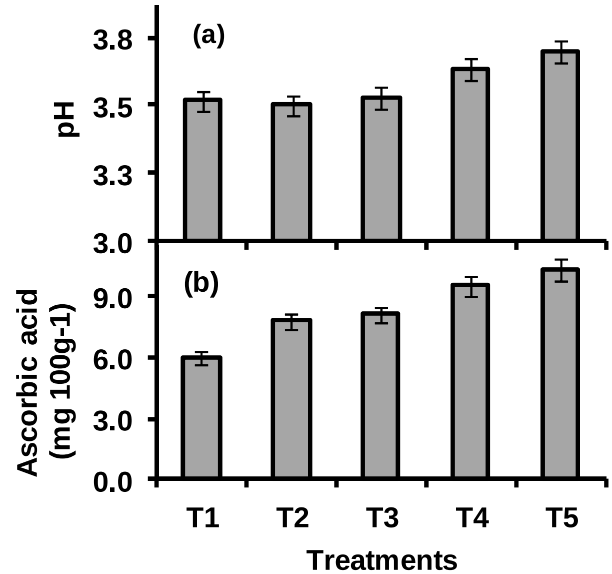
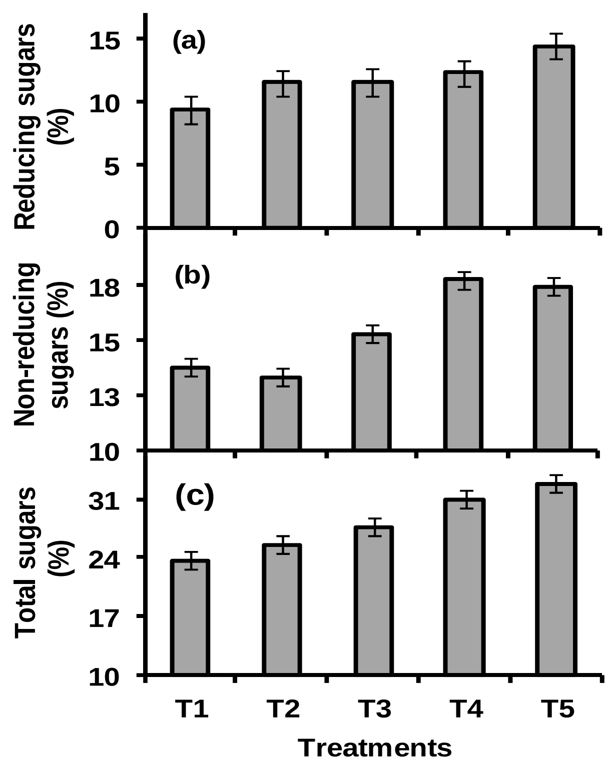


Fig. 5: Effect of foliar application of mixture of amino acids and seaweed extract (0.5 mL L^{-1}) at different growth stages on reducing, non-reducing and total sugars of grapes cv. Perlette. Vertical bars indicate ± SE of means. n = 3 replicate



seaweed extract at different growth stages had a positive effect on vegetative growth, reproductive growth, and berry quality of grapevines. Multiple application of 0.5 mL L^{-1} mixture of amino acids and seaweed extract at flowering + fruit setting + one month after fruit setting stages is quite effective to improve growth and berry physicochemical quality characteristics of 'Perlette' grapes.

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