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Study of agronomic traits and physiological characteristics of lettuce under the applications of salicylic acid (SA)

Noor Fatima¹, M. Sajjad Iqbal¹, Khalid Hussain¹, Zarrien Fatima¹, Khalid Nawaz¹, Noshia Arshad¹, Iqra Razzaq²

¹Department of Botany, University of Gujrat, Gujrat, Pakistan

²Institute of Botany, University of the Punjab, Lahore, Pakistan

Lettuce (*Lactuca sativa* L.) is mainly grown as a vegetable, but it is also as a salad in various countries. Experiments were conducted to evaluate and characterize three lettuce cultivars (Igloo, Red Salad Bowl, and Lolla Rossa) for agronomic, physiological, and yield attributes in response to salicylic acid (SA). SA treatments were 0, 0.5, and 1mM which were applied as foliar spray after 14 days of germination. Results for leaves' fresh and dry weights, shoot length, photosynthetic rate, antioxidant activities, and yield were recorded. Cultivar Igloo showed a more significant number of leaves and considerably higher biomass than the Lolla Rossa and Red Salad Bowl cultivars. Salicylic acid at a concentration of 1 mM enhanced leaves counts to 15. The highest quantity of anthocyanin was noted in Red Salad Bowl which was 0.0047 mg/mL. Results indicated that Igloo has a higher number of leaves and short stems and gives a high marketable yield. Chlorophyll a and b along with the quality of physiological characters were higher as with SA resulting in higher growth. Igloo cultivar was best in growth, but Lolla Rossa had high anthocyanin content and showed the highest carbohydrate content. The Red Salad Bowl showed the highest protein content. Catalase, peroxidase dismutase, and superoxide dismutase activities were also increased by SA. Igloo showed the highest marketable yield, which was 39.69g for fresh leaves. This suggested that Igloo has better leafy growth as compared to Red Salad because consumers prefer those cultivars that have a more significant number of leaves and short stems.

Keywords: Lettuce, Agronomic traits, Antioxidants, Physiological attributes, Salicylic acid

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Khalid Hussain,

Department of Botany,

University of Gujrat, Gujrat, Pakistan

Email: khalid.hussain@uog.edu.pk

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INTRODUCTION

Lettuce (*Lactuca sativa* L.) is well known for its use as salad in various food dressings. It is a leafy vegetable liked all over the world due to the presence of vitamins as well as minerals (Stagnari *et al.*, 2015; Ahmed *et al.*, 2021). Lettuce is a cash crop that contributes to socio-economic development and farmers, especially professional growers earning revenue (Basarir *et al.*, 2022). Even though in Europe, North America, and China lettuce consumption is high as expands to developing countries, lettuce derived from *Lactuca serriola* L wild species probably originated in Egypt (Souza *et al.*, 2017). Various types of cultivars are available in the market, where cultivars with known characteristics should be available (Alneyadi, *et al.*, 2024). In 2017, world production of lettuce was 27 million tons. Several countries and regions are potentially cultivating lettuce on a large scale. Pakistan has 85th rank in lettuce production in the world (Arough *et al.*, 2016). Although production is not so considerable, but still export to other countries like Afghanistan and Maldives is still there.

Nutritionally, people desire the lettuce taste, and the consumption increases day by day. Several meals including green leafy vegetables along with lettuce leaves are considered a main dish (salads). Consumer trends showed their wide use overall and cultivation as a substitute for traditional crops like cabbage and

mint due to high impact (Torrellas *et al.*, 2012). As lifestyle changes, everyone prefers fresh-cut vegetables. People are aware of the importance and quality of freshly produced vegetables for their health in diet pattern, lettuce is an important constituent due to its high nutritional value. It reduces the risk of heart diseases as well as cancer (Nyathi *et al.*, 2018). Policymakers and consumers pay attention to producing indoor Lettuce and lowering the content of nitrate (Ding *et al.*, 2018).

Scientists researched lettuce and how to use it for disease prevention. A case study has shown that consumers of lettuce have an inverse association with colorectal cancer (Kim *et al.*, 2016). The presence of antioxidant compounds polyphenol and vitamin C gives various benefits (Abdalla *et al.*, 2019; Aldhmani, *et al.*, 2022). The presence of antioxidants helps maintain cholesterol levels (Kaur *et al.*, 2023). Hence consumption of Lettuce lowers the risk of cardiac disease (Hefnawy & Ramadan, 2013). The present study shows that providing salicylic acid at a low quantity which is non-toxic to plants increases plant growth. Salicylic acid easily passes through the tissues, organs, and across cells (Ahmed *et al.*, 2022). Salicylic acid is phenolic and an active growth regulator endogenously. Plants protect themselves under stressful conditions with salicylic acid (Farooq *et al.*, 2008b). Different quantitative traits of lettuce demonstrated different responses of each cultivar to the application of salicylic acid.

Biomass production and biochemical parameters of lettuce were enhanced upon the treatment of Salicylic acid as compared to control. Supplements of SA greatly enhance growth factors, the pigment of photosynthesis, the content of prolin (Keshavarz *et al.*, 2020). Some scientists stated that good effects of the application of salicylic acid and the production of flavonoids, soluble carbohydrates, sugars, and antioxidants in lettuce (Khan *et al.*, 2014; Khalil *et al.*, 2022). Supplements of SA greatly enhance growth factors, the pigment of photosynthesis, and the content of soluble protein. (Delavari *et al.*, 2010). In lettuce, SA is produced in low quantity and activates physiological and various biochemical procedures containing germination of seeds, growth of the plant, and photosynthesis (Hayat *et al.*, 2010). By using these hormones plants overcome the effect of Abiotic stress (Hassan *et al.*, 2023).

MATERIALS AND METHODS

The experiment was carried out in the Botanical Garden of the University of Gujrat, Pakistan. Three treatments of SA were sprayed directly to the leaves on three Lettuce cultivars (Igloo, Red Salad Bowl, and Lolla Rossa) after 14 days of germination. Seeds were obtained from Punjab seed Corporation Lahore, Pakistan. There were the following treatments: To=Control, T1=SA (0.5 mM), and T2=SA (1 mM).

The experiment was laid down in a Completely Randomized Design (CRD) with three replicates for each treatment. Morphological traits, physiological parameters (leaf exchange gas, gas exchange through stomata, rate of photosynthesis along with the concentration of carbon dioxide in the intercellular space of leaf, and biochemical parameters (chlorophyll a, carotenoids, anthocyanin, antioxidant SOD, CAT, POD, protein, relative water content, and carbohydrates). Marketable yield was also assessed along agronomic parameters. While leaf area was measured with the help of ImageJ software (Zhang *et al.*, 2020).

Chlorophyll a, b, total, and carotenoids were determined by Arnon (1949) method. Carbohydrates were estimated by the Anthrone method. While protein solubility was measured by Bradford (1979) method. Antioxidant activities were determined using the method of Chance and Maehly (1955). Anthocyanin content was determined by Krizek *et al.*, (1993) method. Relative water content was calculated using Sairam method (2002). Data were subjected to

Analysis of Variance in Minitab (Version: 19.2.0, Coventry, UK).

RESULTS

Morphological Attributes in Lettuce

A highly significant ($P \leq 0.001$) effect of SA was noted, by the flourishing of root attributes (Table 1). The root increased in length (Figure 1). There were highly significant interactions between hormones and cultivars. The maximum root length was found at 1 mM SA in a cultivar of Igloo (Fig. 1). Fresh and dry root weights have considerably become greater with the applications of SA. The results of the interaction among cultivars of lettuce and hormones were highly remarkable and significant (Table 1). The fresh weight of the root was highest in the Igloo with the foliar spray of 1 mM SA. A similar situation of root dry weight happened in the matter of root fresh weight. The utmost root dry weight was weighed in Igloo with 1.0mM of SA (Figure 1).

The effect of SA has a notable significant effect on the progress of the vegetative growth of lettuce i.e. increase in shoot lengths and weights of fresh and dry shoots. There were tremendously significant effects to enlarging the shoot length (Table 1). Interactions among hormones, cultivars, and combinations were also highly significant. The maximum shoot length was measured out in the cultivar Igloo 1 mM of SA (Figure 1). Shoot fresh and dry weights were also significantly and outstandingly increased with the applications of SA (Table 1). Notably significant interactions between all the variables were noted. The supreme weight of fresh shoots was found in Igloo with 1 mM of SA (Figure 1). Cultivar Igloo had maximum shoot dry weight with 1 mM of SA.

The effects of SA were strikingly significant for several leaves and leaf areas per plant (Table 1). Remarkable effects can be seen in the number of leaves by SA treatments for cultivars and the interaction of the hormone x cultivar. The number of leaves is the topmost Igloo with 1 mM SA (Figure 1). The maximum leaf area was noticeable in Igloo both with 1 mM SA (Figure 1). A highly significant ($P \leq 0.001$) effect of SA was noted for root attributes (Table 1). The root increased in length (Figure 1). There were highly significant interactions between hormones and cultivars. The maximum root length was found at 1 mM SA in a cultivar of Igloo (Figure 1).

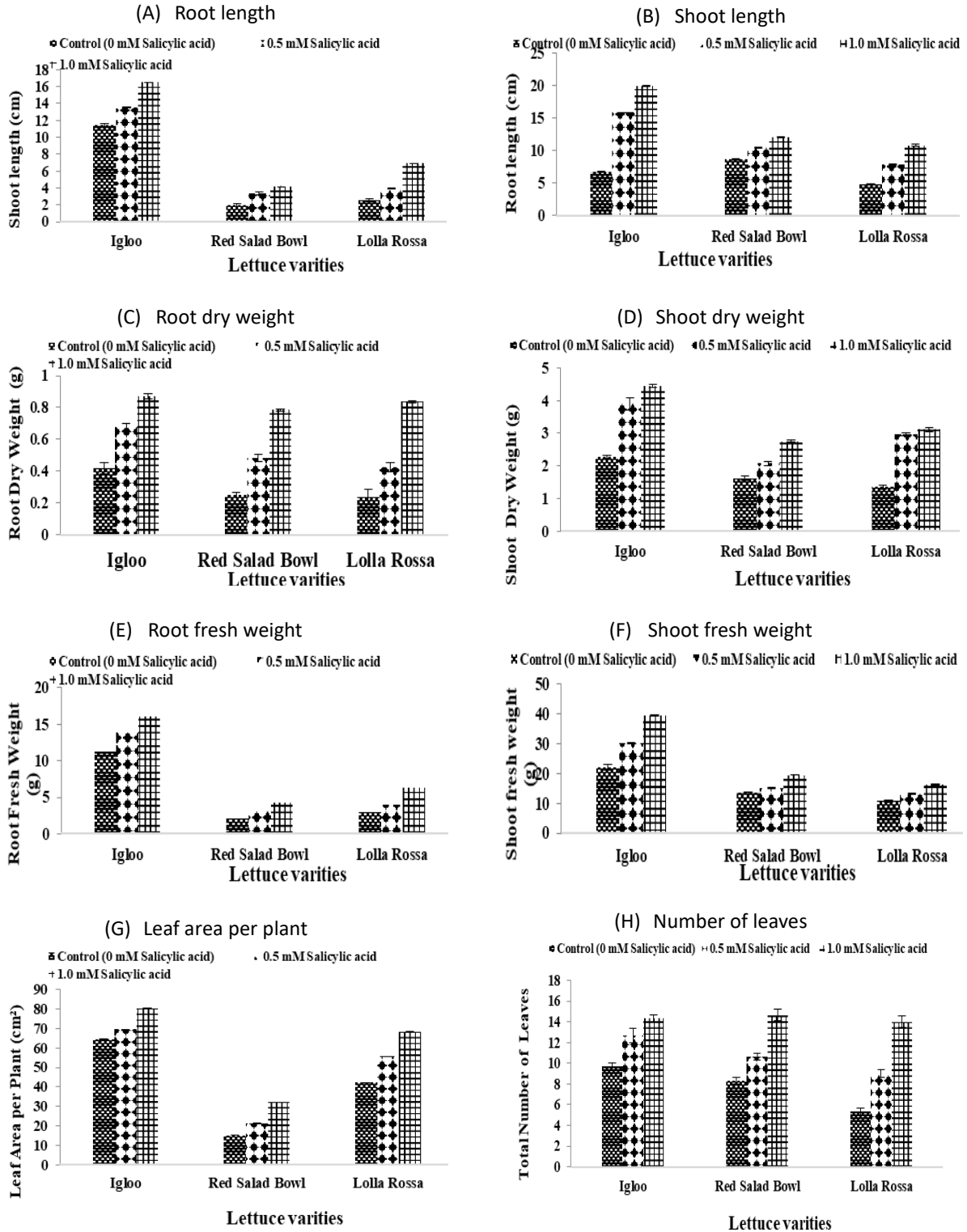


Figure 1. Role of Salicylic acid on morphological attributes of lettuce (*Lactuca sativa*).

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The effects of SA were highly significant on the number of leaves and leaf area per plant (Table 1). There were significant effects for the number of leaves with SA treatments for cultivars and interaction of hormone x cultivar. A higher number of leaves were found in Igloo with 1 mM SA (Figure 1). The maximum leaf area was found in Igloo both with 1 mM SA (Figure 1).

Physiological Activities and Photosynthetic Pigments

The effect of SA is compelling in accessory and chloroplast pigments as seen in lettuce i.e. Chl. a, b, total chlorophyll, and carotenoids (Table 1). These pigments shoot up the use of SA (Figure 2). Meaningful effects for hormones, cultivar, also interaction with cultivar have a major impact. Chl-a, Chl-b, and carotenoids have a considerable impact on all levels of hormones. They all showed significant effects on the cultivar and the interaction of the cultivar and hormones (Figure 2). Most Chl- a, b, and total contents can be seen in the Red Salad Bowl with the treatments of 1mM of SA. Similarly, there were high contents of carotenoid in the Red Salad Bowl with the treatments of 1mM of SA (Figure 2).

Results of (Table 1) stated that the effect of plant growth regulator (SA) is meaningful for every physiological parameter i.e. photosynthetic and transpiration rate, intercellular CO₂ concentration, and stomatal conductance. All variables showed perfect responses toward SA except the transpiration rate which showed a significant response. The rate of photosynthesis was higher in Igloo at 1 mM of SA in contrast to other cultivars (Figure 2). The topmost transpiration rate was noticed in the Red Salad Bowl at 1 mM of SA (Figure 2). Similarly, high intercellular CO₂ concentration was seen in Red Salad Bowl by applying SA at 1mM as compared to Igloo and Lolla Rossa, cultivar Red Bowl Salad showed the highest stomatal conductance at 1mM of SA (Figure 2). Generally, it can be noted that hormone treatment is best in contrast to non-treated for boosting physiological activities.

Antioxidant Activities

Mean squares from ANOVA given in Table-1, perfectly stated the antioxidant activities like catalase and peroxidase, and superoxide dismutase are significantly increased with the application of SA, Varietal response and the interactions were also significant in SOD and POD but it was not non-significant for CAT. The highest CAT activity was observed at 1 mM of SA in Lolla Rossa (Figure 3). Similarly, the highest POD, as well as SOD activity, was observed in Lolla Rossa at 1 mM of SA (Figure 3).

Nutritional Contents in Lettuce

Nutritional contents including total protein, total carbohydrate contents, and anthocyanin in lettuce showed highly significant results in response to hormones (Table 1). Total carbohydrate content and total protein showed a highly significant varietal response, but anthocyanin showed a significant response. The interaction between cultivar and hormone anthocyanin showed highly significant results but carbohydrates and protein content showed significant results. Cultivar Lolla Rossa showed maximum protein content (Figure 3). Carbohydrate contents are elevated in all the cultivars at 1 mM of SA, but Maximum carbohydrate contents were found in Red Salad Bowl at 1 mM of SA. Maximum anthocyanin content was found in Lolla Rossa at 1 mM of SA. It was noted that the 1 mM of SA notably improved the quality traits in all three varieties. (Figure 3).

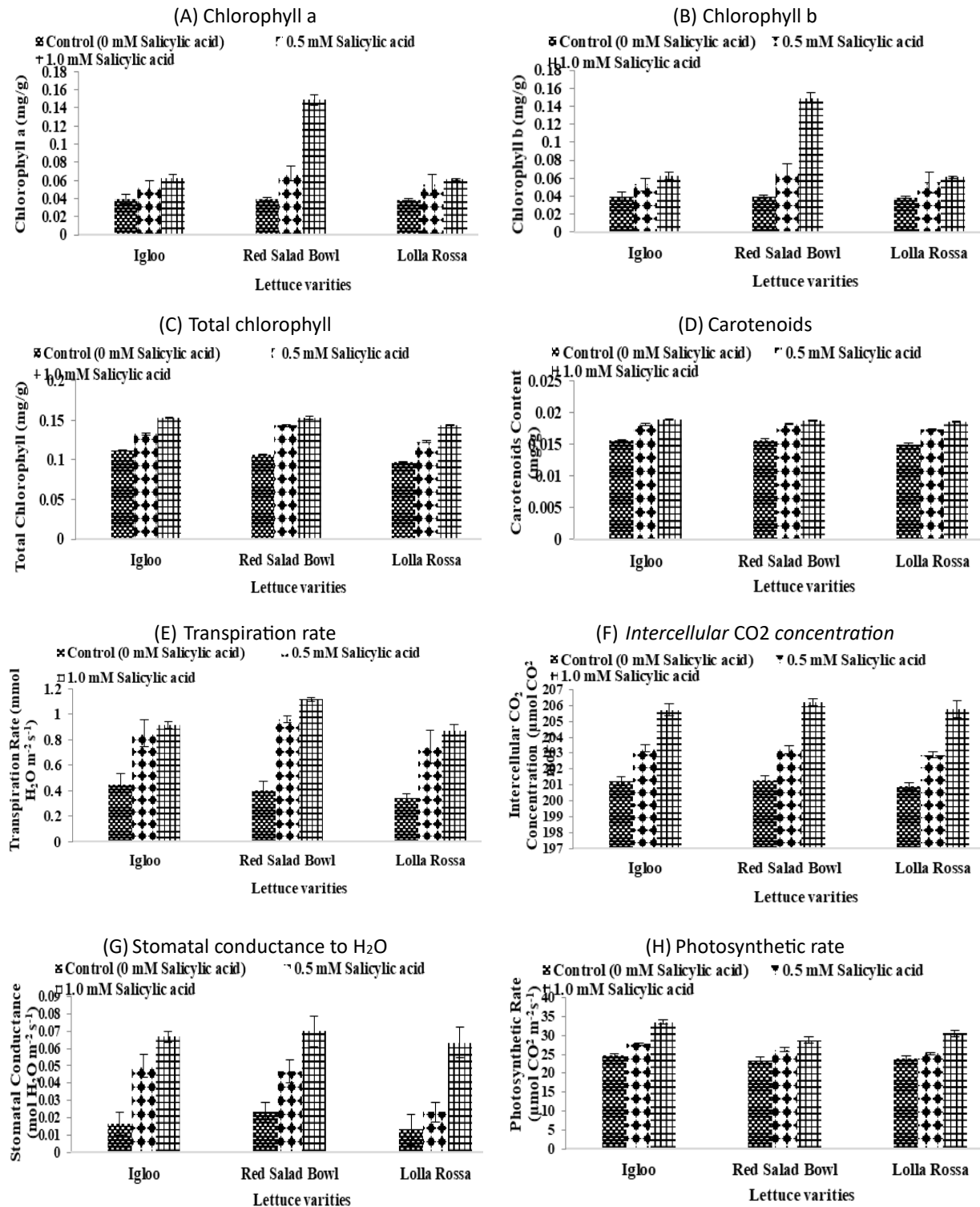


Figure 2. Role of Salicylic acid on Biological and Physiological parameters of Lettuce (*Lactuca sativa*)

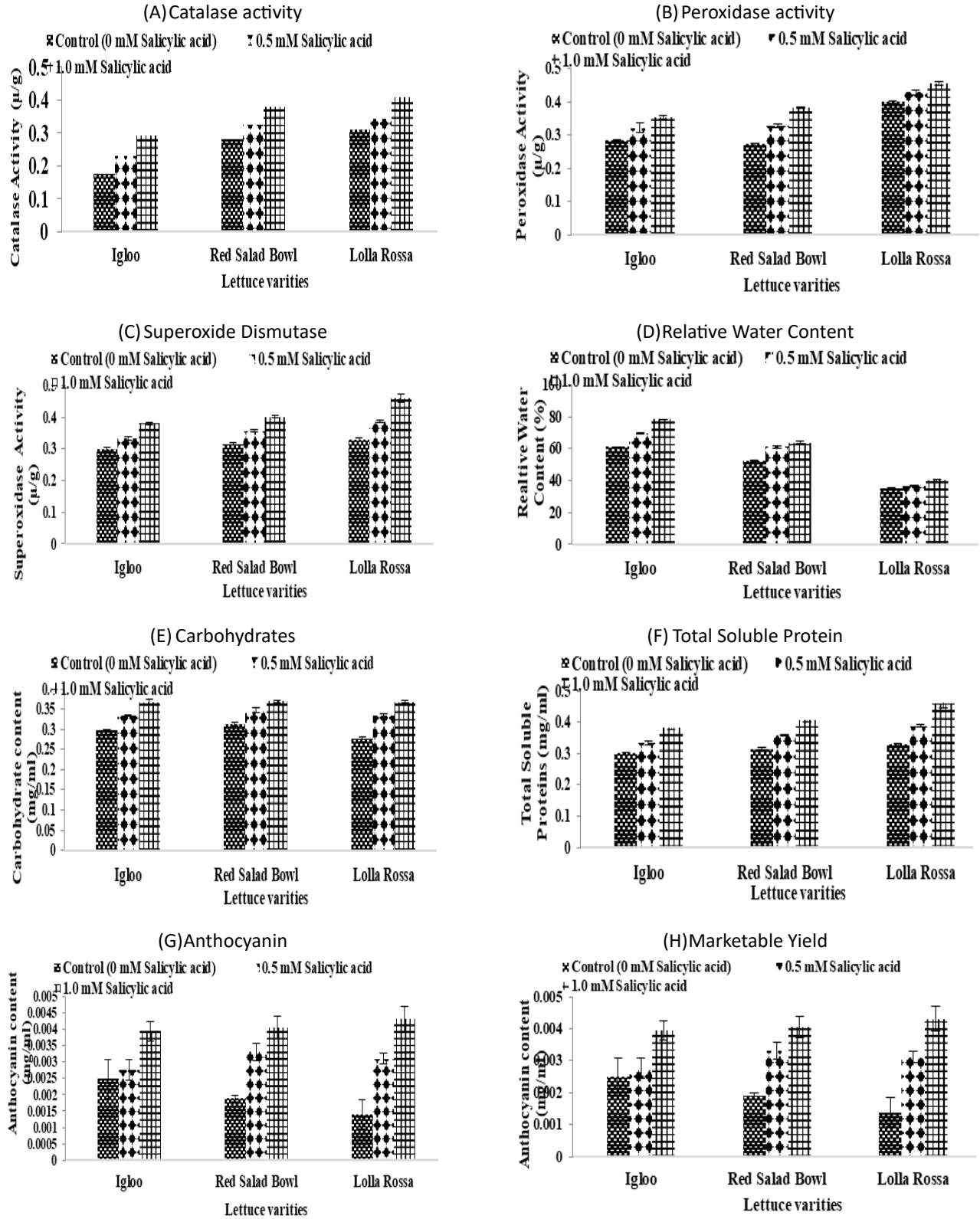


Figure 3. Role of Salicylic acid on Antioxidants, Relative water content, Nutritional and Yield parameters of Lettuce (*Lactuca sativa*).

Yield Attributes

Data concerning yield parameters is listed in Table 1. It must be noted that yield attributes exhibit very outstanding results of SA along with variable interactions. Fresh marketable yield was remarkably increased by the assistance of plant hormones (Figure 3). Igloo showed a high marketable yield as compared to Red Salad Bowl and Lolla Rossa. Similarly, the maximum fresh marketable yield was present in Igloo at 1mM SA.

Pearson Correlation

Correlations among all the attributes are presented in (Table 2). There is a very strong correlation between the traits studied in this investigation, ranging from the highest positive correlation $r = 0.94$ (between the shoot fresh weight and intercellular CO_2 concentration). There is a very high positive association between carbohydrates and stomatal conductance $r=0.94$. Anthocyanin and shoot fresh weight showed a very high positive association because $r=0.90$. Similarly, the highest positive association is shown between yield and root dry weight $r = 0.9$ (Table 2). Total, chlorophyll, and shoot fresh weight showed a highly positive association and $r=0.90$ (Table 2). It means that when total chlorophyll increases the number of leaves also increases in the same direction with the same magnitude. The negative association was seen in very few traits like Chlorophyll a and number of leaves $r=-0.20$ (Table-2). A negative association was also seen between catalase and root dry weight $r=-0.50$ (Table 2). Negative association means when root dry weight increases the catalase activity decreases. SA helped to correlate all the variables positively to enhance the growth, physiological activities, antioxidant activities, photosynthetic pigments, nutritional contents, and fruit yield of lettuce.

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DISCUSSION

Salicylic acid is a growth regulatory hormone that gives good results and rapid growth when it is applied. It enhances the yield of crops significantly. In three subspecies of lettuce, qualitative and quantitative traits-based differences were noted. Between two cultivars of *Brassica rapa* showed the maximum difference based on morphological characteristics such as the maximum length of the leaf (31.6 cm) and the increase in the width of the leaf (13.4 cm) (Jan et al., 2017). The application of salicylic acid improves the qualitative traits of lettuce. Our studies show that the qualitative characteristics of lettuce improve by the application of SA. The application of Salicylic acid on strawberries enhances quality attributes and storage behavior for 8 days (Salari et al., 2003). The morphological characteristics of cucumber-like as the number of leaves, fresh weight of root and shoot, and dry weight of root and shoot enhanced by the application of 0 mM, 0.75 mM, and 1 mM SA. Similarly, in the case of lettuce 15 leaves, a 19.9 cm root length, shoot length of 16.5 cm were noted after the application of SA. The length of the root increased by biochar application (Xing et al., 2017). The length of the root increased by jasmonic acid, but the shoot dry weight was reduced. The length of the root increased by the water content (Farhangi-Abri et al., 2019). The height of stem length increased at the vegetative stage in amaranthus as well as a tomato by application of 3mM of SA because antioxidant response induced by SA which protects from damage (Umebese et al., 2009). A maximum increase in shoot fresh weight was recorded at 200 mg L⁻¹ of SA (Noreen & Ashraf, 2008). The application of 500 ppm of rosemary essential oil enhanced the fresh weight of root as compare to control (Souri & Bakhtiarizade, 2019).

Table 1. ANOVA results for morphological, physiological, antioxidants, biochemical, nutritional attributes, and yield of lettuce due to variety and hormones, and their interactions.

Morphological	Hormone (H)	Variety (V)	H x V	Error	Total
1-Root length	128.73***	90.92***	20.98***	0.01	
2-Root fresh weight	27.77***	297.93***	1.46***	0.02	
3-Root dry weight	0.63***	0.07***	0.00**	0.00	
4-Shoot length	34.05***	305.22***	1.99***	0.05	
5-Shoot fresh weight	215.19***	781.55***	35.83***	0.38	
6-Shoot dry weight	6.88***	4.74***	0.39***	0.02	
7-Number of leaves	34.05***	305.22***	1.99***	0.05	
8-Leaf area	8.66***	5495.85***	26.09***	0.02	
9-df	2	2	4	18	26
Physiological and antioxidants	Hormone (H)	Variety (V)	H x V	Error	Total
1-Chl a	0.00***	0.00***	0.00***	0.00	
2-Chl b	0.16***	0.13***	0.04**	0.00	
3-Total Chl	0.00***	0.00***	0.00***	0.00	
4-Carotenoids	0.14***	0.05*	0.04***	0.00	
5-Photosynthetic rate	112.12***	16.32***	3.57*	1.12	
6-Transpiration rate	0.82*	0.06***	0.01ns	0.01	
7-Intercellular CO ₂ concentration	101.86***	244.71***	33.72**	5.63	
8-Stomatal conductance	0.03***	0.01***	0.00***	0.00	
Df	2	2	4	18	26
Water relative content, Nutritional and yield	Hormone (H)	Variety (V)	H x V	Error	Total
1-Protein content	0.02***	0.00***	0.00*	0.00	
2-Carbohydrate	0.01**	0.00***	0.00*	0.00	
3-Anthocyanin	0.00***	0.00*	0.00***	0.00	
4-Relative water content	284.76***	2450.76***	33.30***	0.67	
5-SOD	0.02***	0.00***	0.00**	0.00	
6-CAT	0.02***	0.03***	0.00ns	0.00	
7-POD	0.01***	0.03***	0.00**	0.00	
8-Yield	215.19***	781.55***	35.83***	0.38	
Df	2	2	4	18	26

The fresh weight of the shoot was increased by the application of SA and SNP (sodium nitroprusside). The highest fresh weight of the shoot was recorded by the application of SA at a concentration of 100 μ M. Besides, the shoot length was increased by the application of SA at 100 μ M. The effect of various concentrations of naphthaleneacetic acid (NAA) (0, 25, 50, 75, 100 mg l⁻¹) increased the root dry weight of weight. The low concentration of bisphenol (BPA) (1.5 mg L⁻¹) has significant effects on root dry weight (Chen *et al.*, 2017).

All parameters that exchanged gas were improved maximum by the application of SA at 100 μ M (Naseem *et al.*, 2020). The remarkable effect of calcium chloride was also noticed in the Snap bean, pepper (El-Tohamy *et al.*, 2006), tomato (Rab and Haq, 2012), cucumber (Kazemi, 2013b), strawberry (Kazemi, 2015), cowpea (Mohamed and Basalah, 2015), and lettuce (Almeida *et al.*, 2016).

The number of leaves increased by application in the Igloo sub-species of lettuce. Al-Abbasi *et al.*, (2015) stated that the application of salicylic acid increases the number of leaves and leaves area of *Zinnia*

elegans L. Another factor is the number of leaves that were increased by the application of nitrogen (120kg N ha⁻¹) in romaine lettuce. By the application of nitrogen (120kg N ha⁻¹) were increased the number of leaves in romaine lettuce (Al-Abbasi, Abbas, & Al-Zurfi, 2015). Morphological character is excellent for screening the best cultivar because it showed a high number of leaves (Fayyaz & Afzal, 2014). Application of 60mg.L⁻¹ appreciably enhanced the number of leaves and total chlorophyll on *Matthiola incana*. Chlorophyll a, b, and total chlorophyll content were found at peak when *L. chinensis* plants were treated with 0.1 mg L⁻¹ BR, which were 80.71%, 98.0%, and 85.57% higher than control (Niu *et al.*, 2016). Maximum quantity (35mg/g) of total chlorophyll obtained from 70% and 85% irrigation level. Under both room as well as high temperature stressed conditions, treatment with 0.1 and/or 1.0 mg L⁻¹ BR proved beneficial regarding enhancement of the chlorophyll as well as carotenoid content (Niu *et al.*, 2016). When SA was applied significantly the enzyme activities as compared to SNP. By application of SA, the activity of SOD was increased 23% in Maize.

Table 2. Qualitative variables of three varieties of Lettuce (*Lactuca sativa*) at different stages

Qualitative variables Parameters	Varieties		
	Igloo	Lolla Rossa	Red Salad Bowl
Stage at which Agro-morphological parameters note	At a stage of fully developed seedling	At a stage of fully developed seedling	At a stage of fully developed seedling
Cotyledon colour	Light green (3)	Light green (3)	Light green (3)
Cotyledon shape	Elliptic (2)	Orbicular (4)	Obovate (7)
Cotyledon trichomes	Absent (0)	Absent (0)	Absent (0)
Anthocyanin-present	Absent (0)	Present (1)	Present (1)
Fully developed stage	At a stage of 10–12 fully developed true leaves	At a stage of 10–12 fully developed true leaves	At a stage of 10–12 fully developed true leaves
Young leaf – position	Erect (1)	Semi Erect (5)	Erect (1)
Young leaf – colour	Green (3)	Purple (99)	Light Green
Young leaf – anthocyanin distribution	Absent (0)	Present (1)	Present (1)
young leaf blade	Entire (1)	Entire (1)	Divided (2)
Young leaf – blade shape in out line	Elliptic (2)	Orbicular (4)	Elliptic (2)
young leaf blade-shape of apex	Rounded (2)	Rounded (2)	Round (2)
Young leaf – anthocyanin distribution	Slight (3)	Diffused on Entire Lamina	Intense (7)
young leaf blade -shape of base	Long attenuate (3)	Long attenuate (3)	Long attenuate (3)
young leaf blade margins	Entire (1)	Crenate (2)	Entire (1)
Young leaf – blade-vertical margin undulation	Intense (7)	Moderate (5)	Intense (7)
Young leaf – trichomes	Absent (0)	Absent (0)	Absent (0)
Young leaf – venation	Pinnate (1)	Flabellate (2)	Pinnate (1)
At stage of maturity	at a harvest maturity	at a harvest maturity	at a harvest maturity
Outer adult leaf– colour	Green (2)	Red and green (5)	Red and green (5)
Outer adult leaf- intensity of colour	Moderate (5)	Intense (7)	Intense (7)
Outer adult leaf- anthocyanin distribution	Absent (0)	Diffused on entire lamina (3)	Diffused on entire lamina (3)
Outer adult leaf anthocyanin intensity of coloration	Absent (0)	Intense (7)	Intense (7)
Outer adult leaf- surface profile	Concave (1)	Flat (2)	Flat (2)
Outer adult leaf blade	Entire (1)	Entire (1)	Divided (2)
Outer adult leaf-entire shape of blade in outlines	Orbicular (4)	Rounded (2)	Spatulate
Outer adult leaf -entire margin of blade	Double dentate (4)	Crenate (2)	Setose dentate (5)
Outer adult leaf-divided- depth of incision	Pinnatifid (5)	Pinnatlobed (3)	Pinnatifid (7)
Outer adult leaf-shape of apex	Subacute (4)	Rounded (2)	Mucronate (5)
Outer adult leaf shape of blade base	Medium attenuate (2)	Short attenuate (1)	Long attenuate (3)
Outer adult leaf-blistering	Moderate (5)	Intense (7)	Slight (3)
Head, leaf rosette	Head	leaf rosette	leaf rosette
Head – formation	Present (1)	Absent (0)	Absent (0)
Harvested part	Small (3)	Small (3)	Medium (5)
Head – shape in vertical section	Transverse elliptic (5)	Absent (0)	Absent (0)
Head – overlapping of leaves	Complete (7)	Absent (0)	Absent (0)
Head – firmness	High (7)	Absent (0)	Absent (0)
Head – weight	High (7)	Absent (0)	Absent (0)
Leaf rosette-position of leaves	Absent (0)	Upright (3)	Medium (5)
Parameters of Stem	Stem	Stem	Stem
Stem – length	Short (3)	Medium (5)	Medium (7)
Stem – fasciations	Absent (0)	Absent (0)	Absent (0)
Stem – anthocyanin	Absent (0)	Present (1)	Present (1)

Table 3. Pearson correlation table between various morphological, physiological, antioxidants, biochemical and yield parameters.

RL	RFW	RDW	SFW	SDW	LA	NL	T	CI	S	P	Chl a	Chl b	Total Chl	C	Carbs	Pro	A	CAT	POD	SOD	RWC	yield	
SL	0.99***																						
RFW		0.58ns																					
RDW			0.56ns																				
SFW				0.66ns																			
SDW					0.84*																		
LA						0.76*																	
NL							0.43ns																
T								0.84*															
CI									0.88**														
S										0.95***													
P											0.88*												
Chl a												0.39ns											
Chl b													1*										
Total Chl														0.68*									
C															0.94***								
Carbs																0.92***							
Pro																	0.79*						
A																		0.83*					
CAT																			0.56ns				
POD																				0.78*			
SOD																					0.80**		
RWC																						0.86**	
Yield																							0.844**

Relationship of parameters and treatments in Lettuce plant. The entire dataset was analyzed using the Pearson approach. The parameters included are RL (Root length), SL (shoot length), RDW (root dry weight), RFW (root fresh weight), SFW (shoot fresh weight), SDW (shoot dry weight), LA (leaf area), NL (number of leaves), T (transpiration rate), CI (intercellular CO₂ concentration), S (Stomatal conductance), P (photosynthetic rate), Chl a (chlorophyll a), Chl b (chlorophyll b), Total chl (total chlorophyll), C (carotenoids), Carbs (carbohydrates), Pro (protein), A (anthocyanin), CAT (catalase), SOD (superoxide dismutase), POD (peroxidase), RWC (relative water content).

The activity of Peroxidase was increased by 16.37% and catalase activity was increased by 70% in a cultivar of Maize named Pioneers (Naseem *et al.*, 2020). Applying 2 and 4% crude oil enhanced the Catalase activity in vetiver grass (Keshavarz *et al.*, 2020). Ozturk *et al.* (2018) stated that applying gibberellic acid at a concentration of 15 mg L⁻¹ significantly increased the activity of antioxidants in jujube fruit. In fenugreek plants by application of 5 mM Ascorbic acid noticeably increased the growth of the plant and antioxidant enzymes (Fatima *et al.*, 2020). Ascorbic acid plays a crucial role in the photosynthetic process and protects *Zea mays* from environmental changes (Loutfy *et al.*, 2020). The effects of presowing seed treatment with different concentrations of cytokinins (kinetin and benzylaminopurine; 100, 150, and 200 mg/L) enhanced photosynthetic capacity (Iqbal & Ashraf, 2005).

Brasinolide (BL) enhanced significantly the rate of germination as well as photosynthetic activity at a concentration of 0, 0.01, 0.1, and 1 mg/L. The rate of respiration and gaseous exchange also increased (Sun *et al.*, 2020). Intercellular CO₂ concentration was improved to maximum by the application of SA at 100µM (Naseem *et al.*, 2020). Anthocyanin was increased by the application of gibberellic acid at a concentration of 80 mg L⁻¹ on pomegranate fruit. Eid and Leila (2006) stated that the application of foliar spray on ornamental flowers enhances the carbohydrates in leaves. indole-3-butyric acid (IBA) and acetylsalicylic acid (ASA) largely increase the total soluble protein in peas (El-Shraiy & Hegazi, 2009). The foliar spray of ascorbate and GA enhanced relative water content in *Thymus vulgaris* L. Stem length is the most crucial factor for the breeding program. The height of the plant is an important character for the breeding of wheat (Karagöz *et al.*, 2007). The number of leaves mainly increases in Igloo, the subspecies of lettuce. Sprayed amino green compounds on the head lettuce give the highest number of leaves. Buyers like to eat leaves and discard stems, so they prefer many leaves (Chung *et al.*, 2011). According to the agronomic characteristics, a greater number of leaves present in lettuce show more salability in the market and have a short diameter. Our results showed that the Igloo subspecies of butterhead lettuce shows a high number of leaves and short stems as compared to the other two varieties. Our results showed the Igloo sub-species of head lettuce shows a high fresh weight of shoot, which is important for yield. The weight of marketable yield was enhanced. High

concentrations (1000 ppm) of carbon dioxide and nutrients increase the biomass of head lettuce (Miyagi & Kawai, 2017). Variations were noted based on Agro-morphological traits to find the best genotype. Morphological character is excellent for screening the best cultivar because it shows a high number of leaves. (Iqbal *et al.*, 2015). Surveys were conducted to gather information to evaluate the preferences of consumers. When breeders have know-how lettuce attracts the consumers by the senses including taste, touch, smell, and sight. People are mostly attracted to my Igloo cultivar due to its green color. They prefer to introduce those cultivars in the market (Kim *et al.*, 2014). Based on the quantitative characterization of three cultivars of lettuce "Igloo" found superior and most profitable cultivar for farmers.

CONCLUSION

The foliar application of plant salicylic acid improved the economically important characteristics of *Lactuca sativa* leaf as the number of leaves and leaf area. These traits have significant importance in lettuce due to economic importance for their marketing. The use of salicylic acid also helped in increasing the size of fresh and dry weight which is supportive in obtaining good marketable yield in the next generation. Thus, the use of salicylic acid in our production system can help growers improve the agronomic traits of lettuce which will ultimately lead to fetching higher prices in the domestic and international markets. RED Salad Bowl and Lolla Rossa showed the highest quantity of anthocyanin. In Lettuce at 1mM SA showed the best result in Igloo.

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