Pak. J. Bot., 49(2): 509-517, 2017.

ASSESSMENT OF GROWTH, YIELD AND NUTRITIONAL VALUES OF PEA (*PISUM SATIVUM* L.) BY FOLIAGE APPLICATIONS OF IAA

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Abstract

Pea (*Pisum sativum* L.) is a commercially adopted rational crop used for the conservative and nutritive purposes. By the foliage applications of IAA, morphological, physiological, biochemical, antioxidant, ions, yield and nutritive values of pea were studied. Pea showed a remarkable response to IAA as its growth, yield and nutritional values were increased. IAA applications increased the morphological and physiological attributes which resulted higher yield of the crop. High concentrations of K^+ and N were also noted. CAT activities were increased which resulted better balance in plant metabolism and POD activities were reduced that increased the defense mechanism of plants. High rate of photosynthetic and its pigments concentrations helped to produce the higher crop yield. High contents of protein, carbohydrates and fiber were noted in fresh seeds that was a sign of better nutritional composition. It was concluded that increased morphological, physiological and changes in ionic contents and enzyme activities can be helpful to increase crop yield and nutrition composition of pea for better income and good diet. These changes can be used as indicators in pea varieties to predict better yield and nutritional values.

Key words: IAA, Yield, Nutrition, Pea, Growth.

Introduction

Pea (*Pisum sativum* L.) is the most important leguminous crop which is grown and used for multiple advantages on world level (Macas *et al*., 2007). As a forage crop, it is highly important for livestock and its fodder is used for enhancing effect of milk production due to the presence of leutins (Elzebroek & Wind, 2008). Peas and other legumes are highly appreciated in rotation of crops affected due to disease and pest attacks, nitrogen stimulation, for betterment of microbe's variation and to reduce their dominance in soil, to improve soil texture and improvise soil water conservation (Lupwayi *et al*., 1998; Biederbeck *et al*., 2005; Chen *et al*., 2006). Pea is also used as green manures and cover crops due to their growth and nitrogen fixation ability in the soil through their nodule formation (Ingels *et al*., 1994; Clark, 2007). Pea is highly rich in production of amino acids, lysine and tryptophan as compared to other crops by having 21-25% more traces of carbohydrates and other nutrients (Kent & Endres, 2003). Pea is grown throughout the world for diverse uses as food and fodder. Although it has been long recognized as a world's third significant crop, its production has been rather low for a long time with low cultivated area (Murtaza *et al*., 2007; Podleśna *et al*., 2015).

Plant hormones used for most purposes on different plant growth levels and many of these regulators have interacted in order to observe the final effect. The plant growth regulators are compounds that in minor amounts modify the physiological processes of plants and ultimately alter the yield and quality (Sajid *et al*., 2016). Indole-3-acetic acid (IAA) is the main auxin in plants which controls essential physiochemical pathways i.e. cell elongation and cell division, differentiation on tissue level, phototropism and geotropism effects (Hussain *et al*., 2011). Indole 3-Acectic Acid (IAA) is a naturally existing auxin. Auxins are considered to be the most important hormone for enhancing growth and organized development in plant tissue and organ cultures (Evans *et al.,* 1981; Vasil & Thorpe, 1994). Artificially applied IAA interrelates with endogenously present plant hormones. Synthetic exogenously applied IAA act like natural plant regulator by introducing many regulatory as well as some inhibitory parameters which are helpful for the study of foliar application of IAA and its transportation into the plant (Davies, 1995).

IAA showed influence on pea plant growth by enlarging leaves and increasing photosynthetic activities in plants. During the syntehtic process, IAA also activates the translocation of carbohydrates (Awan *et al.,* 1999). Under stress conditions, significantly decreased IAA concentration in leaves is observed and plants shows less resistance against external fluctuations (Xie *et al*., 2003). It has also reported that exogenous application of indole acetic acid can overcome adverse effects of stress by increasing the ROS generation because pea plant is highly rich in proteins and fibers (Chakrabarti & Mukherji, 2003). IAA (indole-3-acetic acid) is major auxin involved in many physiological processes in plants and stimulates cell elongation, differentiation of vascular cambium and promotes flowering (Khan & Chaudhary, 2010). It is also reported that under stress conditions IAA decreases injuries to plant by reducing the osmotic pressure and protect turgidity of cell in pea. By this action, plant shows a significant increase rate in the production of carbohydrates, amino acids and some insoluble proteins which plays important role in growth regulatory attributes (Agarwal & Gupta, 1995). Similarly, Indole acetic acid significantly increased all the growth parameters as shoot and root lengths, shoot fresh and dry weights, number of leaves and yield per plant in chaksu and scurf pea (Hussain *et al*., 2010; Hussain *et al*., 2011).

In the view of above mentioned literature, it was noted that pea crop production has been low with low cultivated area for a long time with reduced nutritional values, so this study were designed to find the efficacy of IAA that can be helpful to enhance the yield and nutritional values of pea by studying different changes in morphochemical, physiological and biochemical attributes in different pea varieties.

Materials and Methods

Experiment was conducted at University of Gujrat and PCSIR labs, Lahore, Paksitan during 2014-15. The seeds of three varieties of pea (i.e. New Zealand Green Grass, Novada, Selected India) were collected from Arain Seed Store, Lahore, Pakistan.Ten seeds were sown per pot, containing 8 kg dry river sand. Hoagland full strength solution was applied with the interval of 15 days (Hoagland & Arnon, 1950). After 7 days of sprouting, plants were thinned to four plants per pot. Three levels of treatment of Indole Acetic Acid were applied that were:

T_0: 0 ppm of IAA (Control)
T_1: 100 ppm of IAA
T_2: 150 ppm of IAA

Foliar treatment was applied after 14 days of sprouting. Treatments were applied in single dose foliage application. High levels of IAA were selected as there was no use of high concentration of IAA on pea in previous published literature. Experiment was laid down in Completely Randomized Design (CRD) with three replicates containing three pots for each treatment.

Plants were harvested after 35 days of treatment for the study of morphological (shoot and root lengths, shoot and root fresh and dry weights, leaf area), physiological (Chlorophyll, caroteniods, Net CO2 assimilation, transpiration rate, sub stomatal CO2 concentration, stomatal conductance and water use efficiency) and biochemical (antioxidant, proteins, carbohydrates, fiber)and ionic concentrations. Yield parameters were calculated at maturity of the crop. Shoot and root lengths (cm) were measured with the help of scale and weights (g) were taken by electrical balance for both shoot and root separately. Dry weight (g) was taken after drying in oven at 65°C for 4 days. Chlorophyll a, Chlorophyll b, total chlorophyll and carotenes were measured by the method described by Arnon (1949). Fresh healthy leaves were selected to find the gas exchange characteristics using LCA-4 ADC portable open system infrared gas analyzer (IRGA) (Analytical Development Company, Hoddesdon, England). $K^+$and Na^+ concentrations were measured with the help of flame photometer (Jenway, PEP-7) after H_2SO_4digestion. Activities of Catalase (CAT) and Peroxidase (POD) were measured using the method of Chance and Maehly (1955). N determination was proceed by Kjeldahl method. Protein contents were measured by the procedure given by Lowery *et al.* (1951) method. Total carbohydrates were determined by Anthrone Method.Analysis of variance was computed using the COSTAT computer package and mean values were compared by Duncan's New Multiple Range test (DMRT) at 5% level of probability which was used to test the differences among mean values following the method of (Steel & Torrie, 1980).

Results

There were following results by pea plants under the application of IAA.

Morphological attributes: From the results obtained for morphological characters, it was noted from ANOVA that the effect of IAA was highly significant on all growth parameters including shoot length, shoot and root fresh and dry weights and leaf area except root length (Table 1). However variations among varieties and IAA interactions were non-significant except in the case of root length and root dry weight. Fig. 1 (A-E) showed that growth increased at all levels of IAA. IAA concentration 100 ppm had better effects as compared to high concentrations (150 ppm of IAA).

Physiological studies: Analysis of variance for data regarding physiological attributes is given in table 2. Overall effect of IAA was significant on physiological parameters except transpiration rate and sotamatal conductance. Effect of IAA was highly significant on photosynthetic pigments (chl. a, total chl and carotenoids) and it was significant in case of chl. b (Table-2). Chlorophyll contents increased with increase in the level of IAA (Fig. 2 A-C). It was also noted that there were variations among the three varieties for chl. contents.

Table 1. Means squares (MS) fromthe Analysis of Variance (ANOVA) for growth attributes of Pea (*Pisum sativum* L.) under the applications of IAA.

Source	df	MS of shoot length	MS of root length	MS of shoot fresh weight	MS of shoot dry weight	MS of root fresh weight	Ms of leaf area/plant
Main effects IAA	2	516.14***	6.122^{ns}	725.455***	3.293**	8.223***	14.779**
Variety	2	8.074ns	10.398*	10.534ns	4.905ns	2.693*	2.922ns
Interaction IAA x Variety	4	12.764ns	11.258*	28.715ns	8.863ns	1.505ns	3.824ns
Error	18	27.56	2.835	26.229	4.949	0.731	1.491
Total	26						

Table 2. Means squares (MS) from the analysis of variance (ANOVA) for physiological attributes of Pea (*Pisum sativum* L.) under the applications of IAA.

Source	df	MS of Chl. a	MS of Chl. b	MS of total chl.	Ms of carotenoids	MS of photosynthetic rate	MS of transpiration rate	MS of stomatal conductance	MS of water use efficiency
Main effects IAA	2	0.016**	1.070*	16.650**	37.258*	2.670**	1.371ns	0.002ns	1.396**
Variety	2	0.053*	0.547ns	3.551**	2.548ns	28.051*	0.673ns	0.008ns	29.579**
Interaction IAA x Variety	4	0.035*	0.067ns	3.059*	1.641ns	8.220*	1.011ns	0.003ns	3.590ns
Error	18	0.003	0.301	6.235	14.534	6.391	0.630	0.003	2.749
Total	26								

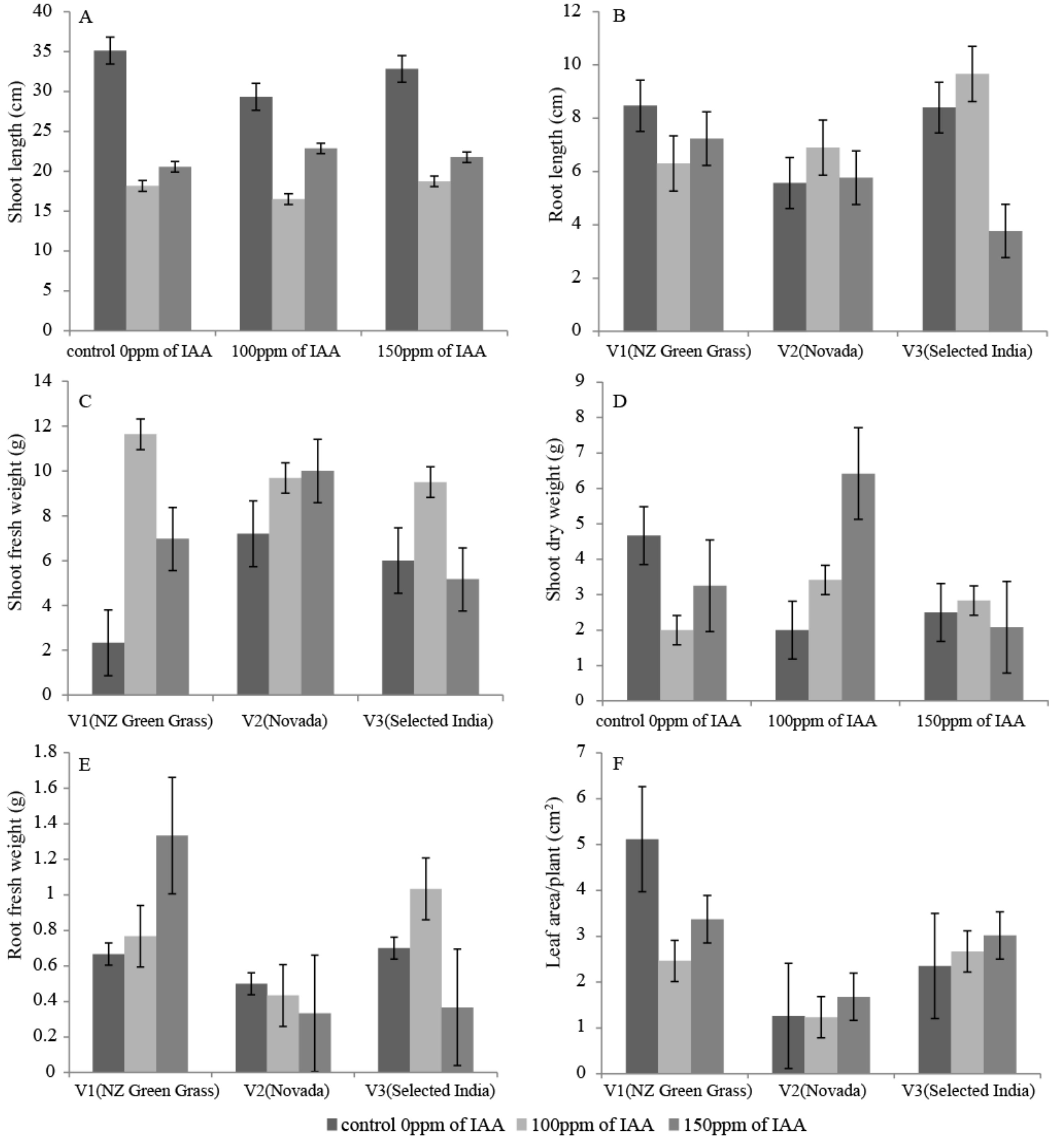

Fig. 1. Effect of different levels of IAA on morphological attributes of Pea (*Pisum sativum* L.).

Antioxidant activities: Data regarding antioxidant activities is given in table and Fig. 3. It was noted that IAA had highly significant effect on CAT and POD activities. CAT activities were increased while POD was reduced under IAA applications. Results for CAT were highly significant. There were also significant variation among varieties and interactions (Table 3). Maximum CAT activity was present in variety Selected India with 100ppm of IAA (Fig. 3). Among varieties, lowest CAT activities were noted in Novada variety. In case of POD activities, there were also highly significant results (Table-3). Maximum reduction in POD activities were noted in variety Novada (Fig.3).

Ionic concentrations: Effect of IAA was significant on all the ionic concentrations in pea i.e. Na^+, K^+ and N both is roots and shoots (Table 4). In case of N in shoots, the results were highly significant. Variations in varieties were also found significant to highly significant except in K concentrations. Na concentrations were reduced with applications of IAA except in NZ Green Grass (at 150 ppm IAA) and Novada (at 100 ppm IAA). On the other hand K and N concentrations were increased with the applications of IAA except in variety NZ Green Grass (Fig. 4).

Yield and nutritive attributes: Yield and nutritive attributes highly significantly increased with the applications of IAA (Table 5). Number of pods/plant, seeds per pod, dry seed weight (total yield), protein, carbohydrates and fiber contents increased with the increase of IAA applications (Fig. 5). Maximum yield was obtained from variety Selected India at 150 ppm of IAA (Fig. 5C).Protein contents in seeds increased with the increased of IAA concentrations. High protein contents were noted in variety Selected India. Variety NZ Green Grass showed non-significant results for protein contents (Fig. 5D). Similar pattern of results were noted in case of total carbohydrates and fiber contents in seeds. Increase in nutritive attributes resulted increase in nutritional value of the pea due to foliar applications of IAA.

Discussion

An increase rate in the number of branches was exhibited in pea under IAA foliar application which caused an enhancement in the mass of shoot weight due to the formation of multiple branches (Malik & Saxena, 1992). Cell enlargement was stimulated by IAA appliance on reproductive and vegetative growth levels of other plants i.e. wheat (Singh & Rathore, 1998). It was also proved that for the development and emergence of lateral roots, auxin reacts as an inhibitory reagent and it effects the overall formation of roots by showing retarded growth (Casimiro *et al.*, 2001; Bhalerao *et al.*, 2002; Benkova *et al*., 2003). Similar effects were shown for dry weight in the shoot of cowpea by increasing dry weight under foliar treatment with IAA on vegetative stages (Khalil & Manndurah, 1989).Many scientists observed that better influence of IAA on leaves having maximum number and showing an enlargement in leaf area (Das *et al.*, 1992; Mishra *et al.*, 2000; Nandhini *et al*., 2001).

Lim *et al.* (2003) observed the plant photosynthetic apparatus under Jasmonates and ethylene but auxins are helpful in delaying the leaf damage and supporting the plants chlorophyll rate to improve. A result under plant regulators also revealed a reduction phase in chlorophyll rate in those genes which only express under higher light availability (Wingler *et al.*, 1998). Similar results were found under the application of the phytohormones in pea by Ahmed *et al*. (1989) because chlorophyll contents showed significant reduction but the seeds showed an improved storage of these contents under study when IAA was applied with ABA.). In beans, a number of evidences are recorded according to which IAA and other hormones are responsible for keeping a balance in photosynthesis and water relations (Munns, 2002). IAA is also responsible for stimulating growth in the plant under drought conditions (Ahmadi & Baker, 2001).

Antioxident activities are similar to the readings recorded by Synkova *et al.* (2004) which showed that foliar application of IAA has an effect on plants by activating the enzymes and improving their activity within the cell under IAA. Thus, these activities are helpful in maintaining the balance in the plant metabolism (Tognetti *et al*., 2012). The reduction of POD activity was noted due to the less passage through cell wall when the dose of Indole Acetic Acid increased because IAA affected POD activity by stopping or initiating the manufacturing ability (Lagrimini, 1996; Klotz & Lagrimini, 1996). A difference was also noted with respect to IAA oxidation by Gonzale *et al.* (1999) and some antioxidant enzymes are also considered responsible for the breakdown of Plant hormones (Szechyńska-Hebda *et al.*, 2007). Peroxidases are proteins which protect plant's cell wall being hardly rich in lignin components by their decomposition (Patel & Thaker, 2007). It is also considered that plant peroxidases have some traces of IAA-oxidase activity which play an essential role in auxins catabolism and alternation of mechanical nature of cell wall (Cosio & Dunand, 2009). The increase in IAA levels in different varieties of pea increases the basic components necessary for the defense of cell wall from pathogen attack by extracting enzymes i.e pectin, cellulose and protein (Agrios, 2005).

It was proved that in the comparison of sodium and potassium internal stimulation, the rate of K^+ ions showed higher ionic storage in the roots of maize under IAA foliar application (Bohra & Dörffling 1993; Botella *et al.*, 1997). A number of experiments are conducted on sugarcane in Brazil to check the nitrogen metabolism under the foliar application of IAA which showed an increase in the nitrogen contents leading its percentage from 60% to 80% (Lima *et al.*, 1987).

In many studies, an enhancement factor in protein was also noted in the plants of *Vicia faba, Cladophora dalmatica, Enteromorpha intestinalis, Ulva lactuca, Corollinamediterranea, Jania rubens, Pterocladia pinnata* and *Cassia absus* under the application of growth regulators (Hussain *et al*., 2011; El-Sheekh & El-Saied, 1999). Solubility of proteins increased differently on different level of treatment (Blackman *et al.*, 1992). Synthetic IAA affects the ratio and concentration of already present auxin by changing it directly by producing enzymes (Maeda & Thorpe, 1979). It is considered that auxin are found binding to a variety of proteins for the growth and cell division in the plants. These proteins are responsible for the processing of many metabolic cycles contributing in plant's metabolism and act as receptors for physiological actions taking place within the cell (Venis & Napier, 1991). By increasing the ratio of IAA, concentration of naturally free auxins also increase which contribute in the cellular activities i.e. anabolism, catabolism, transport, and conjugational activities (Bandurski *et al.*, 1995). Baraich *et al.* (2016) found increased yield by the application of different nutrients in sunflower.

Pea is highly rich in production of amino acids, lysine and tryptophan as compared to other crops by having 21-25% more traces of carbohydrates and other nutrients (Kent & Endres, 2003). IAA application shows direct impact on the yield by increasing the rate of productivity of pods per plant and a remarkable increase in the weight of pods and seeds (Emongor, 1997). Being sensitive to biotic and abiotic conditions pea undergoes endogenously in reduce rate of productivity which may overcome by the providence of growth regulators like IAA (Santana *et al.*, 2009). IBA, a synthetic auxin, is also used commercially for improving annual yield but unlike IAA it shows a stunted growth in the development of lateral roots (Nagel, 2001). However, application of IAA can increase the grain filling phenomenon under control conditions in pea (Ray & Choudhuri, 1981). Similarly, IAA significantly increased number and weight of pods and seeds per plant in cowpea (El-Saeid *et al*., 2010).

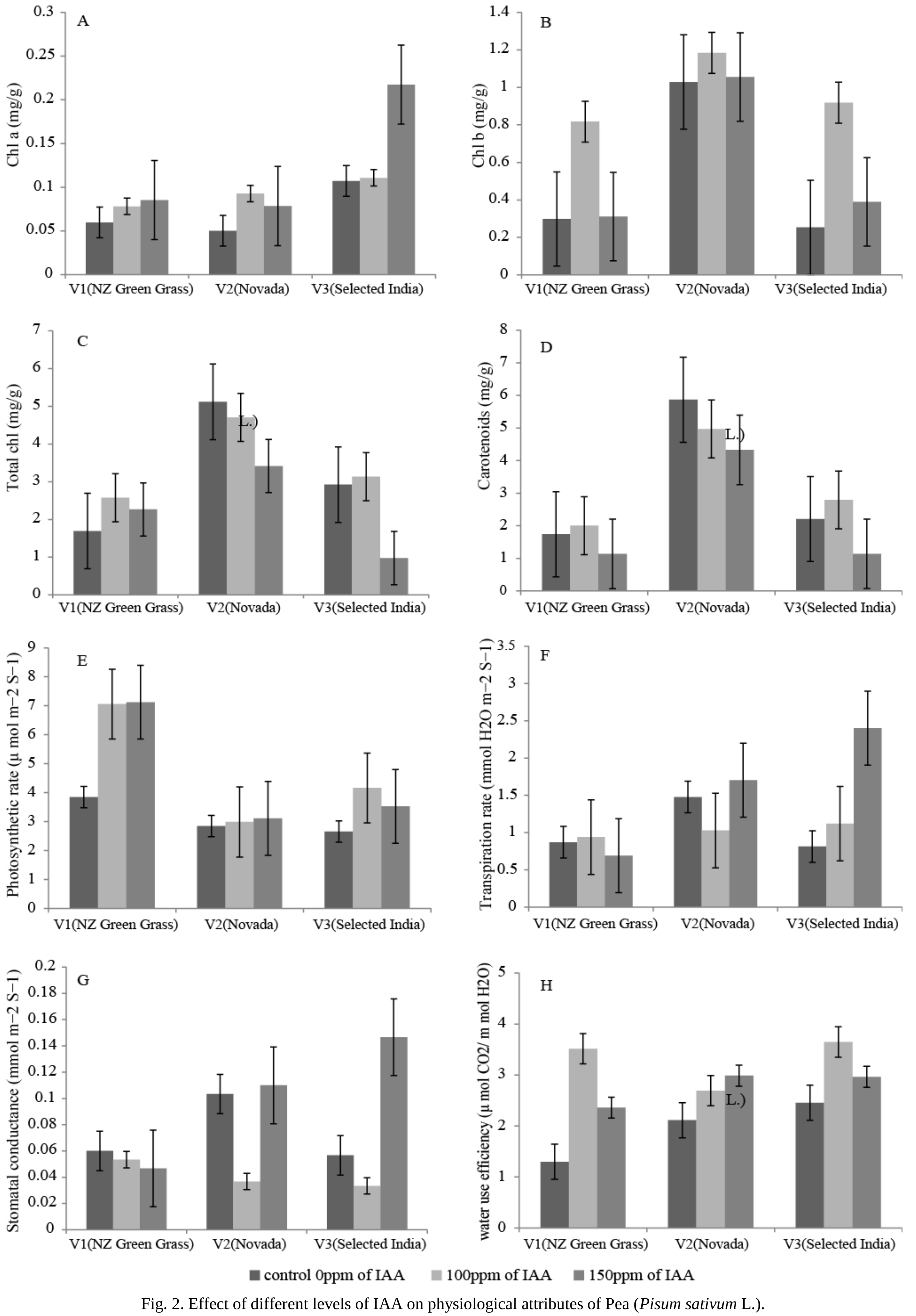

Fig. 2. Effect of different levels of IAA on physiological attributes of Pea (*Pisum sativum* L.).

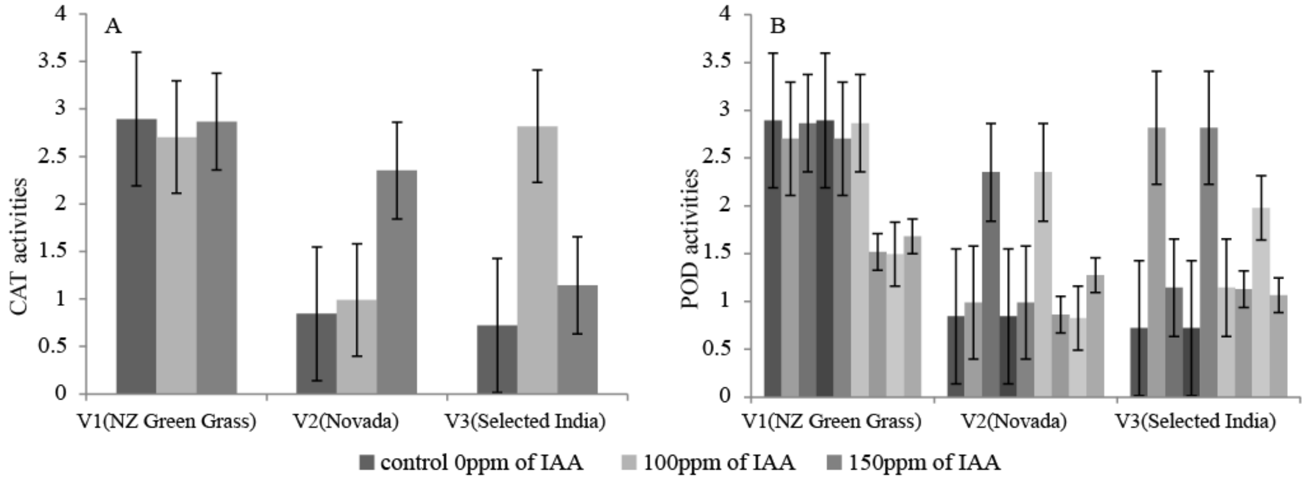

Fig. 3. Effect of different levels of IAA on antioxident activities of Pea (*Pisum sativum* L.).

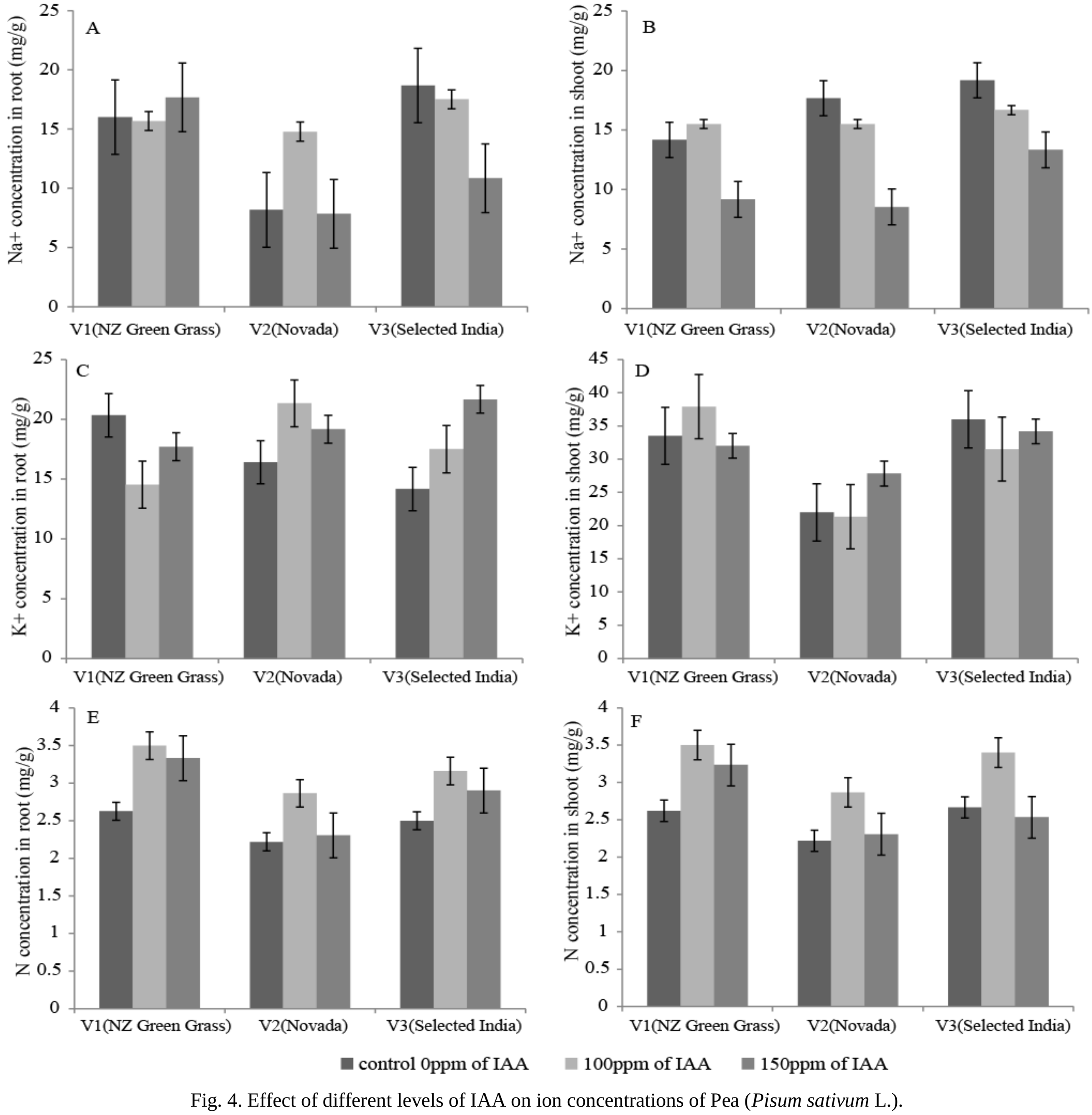

Fig. 4. Effect of different levels of IAA on ion concentrations of Pea (*Pisum sativum* L.).

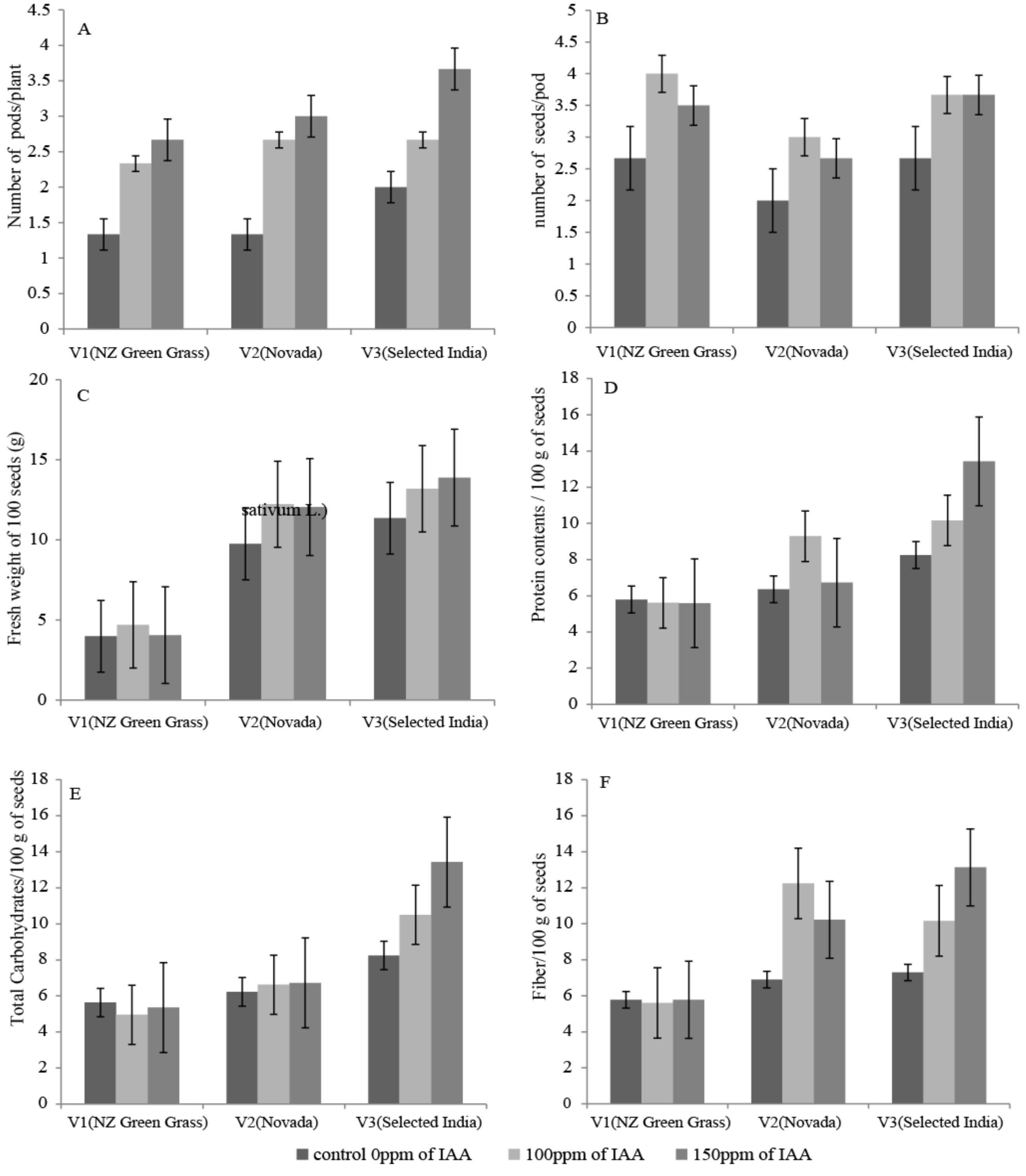

Fig. 5. Effect of different levels of IAA on yield and nutritive attributes of Pea (*Pisum sativum* L.).

Table 3. Means squares (MS) from the analysis of variance (ANOVA) for antioxident activities of Pea (*Pisum sativum* L.) under the applications of IAA.

Source	df	MS of CAT activities	MS of POD activities
Main effects IAA	2	5.460***	0.789**
Variety	2	1.006***	0.159*
Interaction IAA x Variety	4	2.707***	0.418*
Error	18	0.021	0.328
Total	26		

Conclusion

It was concluded that increased morphological, physiological and changes in ionic attributes and enzyme activities can be helpful to increase crop yield and nutrition composition of pea for better income and good diet. These changes can be used as indicators in pea varieties to predict better yield and nutritional values.

Table 4. Means squares (MS) from the analysis of variance (ANOVA) for chemical attributes Pea (*Pisum sativum* L.) under the applications of IAA.

Source	df	MS of Na^+ conc. in roots	MS of Na^+ conc. in shoot	MS of K^+ conc. in roots	MS of K^+ conc. in shoot	MS of N conc. in root	MS of N conc. in shoot
Main effects IAA	2	102.281*	28.457*	8.873*	328.707*	0.018*	0.079**
Variety	2	33.803*	114.41*	20.605ns	2.918ns	0.776***	1.289**
Interaction IAA x Variety	4	34.574ns	6.527ns	33.730ns	39.507ns	0.021**	0.087**
Error	18	28.484	40.450	25.562	60.125	0.004	0.016
Total	26						

Table 5. Means squares (MS) from the analysis of variance (ANOVA) for yield and nutritive attributes of Pea (*Pisum sativum* L.) under the applications of IAA.

Source	df	MS no. of pods/plant	MS no. of seeds/pod	MS of total yield/plant	MS of protein contents	MS of total carbohydrates	MS of seed fiber
Main effects IAA	2	1.444**	1.814***	0.344***	1.345**	2.876**	1.843**
Variety	2	5.778*	2.814**	0.663*	0.987**	1.321**	1.212**
Interaction IAA x Variety	4	0.056ns	1.259*	0.268*	0.369*	0.976*	0.876*
Error	18	1.333	1.592	0.365	0.878	1.334	1.212
Total	26						

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(Received for publication 20 December 2015)