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ORIGINAL ARTICLE

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Effect of Soil Salinity on the Nodulation and Leghaemoglobin in two Variables of (*Pisum sativum* L)

B.K. Verma*, Pravendra Gangwar and K.A. Varshney

[1] *P.G. Department of Botany, Gangasheel Mahavidyalaya, Nawabganj, Bareilly – 243007 (U.P.) INDIA*

*E-Mail:***drbirendrakverma@gmail.com**

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Considerable work has been done on the root nodules and leg-haemoglobin contents of different leguminous plants (Wilson, 1970; Singh *et al*, 1973; Balsubramanian and Sinha, 1976a, b; Kumar and Garg, 1980; Batra and Bhardwaj, 1981; Gupta and Varshney, 1999). In the present study, an effort has been made to assess the effect of soil salinity on the growth and leg-haemoglobin content of nodules in two varieties of field pea (*Pisum sativum Linn.*). The study was conducted in soil beds (size 1 m^2) under natural conditions, in Botanical garden of Gangasheel Mahavidyalaya, Faijullapur, Nawabganj, Bareilly (U.P.). Three replicates were taken for each treatment (control EC 8 CE 8) of both varieties (Var. Pant pea 42 and Pant pea-66). The seeds were sown with gap of equal distance (1 ft.) in soil bed. The result indicates that variety P**ant pea-42** is more salt tolerant than **Pant pea-66**. The number and size of nodules decrease with increase in salinity level in both varieties at all stages of growth (leafy, flowering and maturity). Leg-haemoglobin decreases with the increase level of soil salinity. This study concludes that soil salinity decreases the number of nodules, size of nodules and leg-haemoglobin contents of **Field pea** (*Pisum sativum Linn.*).

Key words: Field Pea (Pisum arvense Linn.), Root nodules, Leg haemoglobin and salinity.

The responses of plants, to the severities of their environment, have occupied the attention of human long before the beginnings of science of biology (Levitt, 1980).

The recent years, biologists have adopted the term “stress” for any environmental factors potentially unfavourable to living organisms and stress resistance for the ability of the pulse crop plants to survive under the unfavourable conditions. The living organisms may show a physical strain or change (eg., cessation of cytoplasmic streaming) or a chemical strain a shift in metabolism.)

If either strain is sufficiently survive, the organisms may suffer a permanent set i.e., injury or death. Living organisms are adaptable. They are therefore, capable of changing gradually in such a way as to decrease or prevent a strain when subjected to stress. This adaption may be stable, having arisen by evolution over a large number of generations, or unstable, depending on developmental stages of plant and the environmental factor to which it has been exposed.

The growth of pea plant growth processes, seed germination, seedling emergence and early seedling growth are considered critical for raising a successful crop as these indirectly determine the crop stand, density and consequentaly the yield of resultant crops (Gelmond, 1978). Gupta (1977) has stated that the first effective increment of salinity for the given crop retards the rate of germination with little or no effect on the ultimate number of seedlings that emerge out. However, at higher level, salinity not only delays but also decrease the final germination percentage. Such effects may be osmotic or lonic resulting in reduction in final percentage of germination (Kent and Lanchli 1985; Hampson and Simpson, 1990; Dash and Pande, 2000).

Soil salinity imposes a significant limitation on productivity by adversely affecting the host plants, root nodule bacteria, symbiotic development and N_2 fixation capacity (Yousef and Sprent, 1983; Zehran and Sprent, 1986). Nodules of leguminous plants in which the atmospheric diatomic nitrogen is fixed by the symbiotic association of ***Rhizobium*** are the established vehicles of biological nitrogen fixation. Nodulation is a very complex biological mechanism which is dependent on the genotypic system of both the bacteria and the host and depends upon the growth and survival of Rhizobia, availability of the sites for infection with ***Rhizobium*** and growth of infected host cell.

In addition to aforesaid physiological parameters, salinity affects water and ionic status in plant cells (Hasegawa *et al.,* 2000). Under salinity stress conditions, the uptake of nutrient elements by pea crop plants is generally affected. Salinity stress may promote nutrient uptake by plant species by affecting the mobility of a nutrient within the plant or by increasing the nutrient requirement by plants in the cells informed that the uptake of Fe, Mn, Zn and Cu generally increases in pea crop plants under salinity stress.

The detrimental effects of NaCl on the nutrition of pulse crop like bean plants are reflected in higher concentration of Mn in roots and Fe and Mn in leaves and Fe in fruits (Carbonell-Barruchina *et al.,* 1998). Erdal *et al.,* (2000) determined that high salinity increases Mn, Cu and Fe contents of cucumber seedlings.

The mechanism of salt tolerance of cultivated crop species that differ considerably in tolerance to salinity generally range from restricted ion uptake and translocation into the shoot to structural metabolic changes that decrease salt injury.

MATERIALS AND METHODS

Source & sterilization of seeds

The certified seeds of 10 germplasms of field pea *Pisum sativum Linn.* were procured from G.B. Pant University of Agriculture and Technology Pantnagar (Uttarakhand), IIPR Kanpur Uttar Pradesh and IARI New Delhi (India). All glassware were cleaned with liquid detergent followed by a dip in chromic acid overnight. Seeds were sterilized by dipping them in 1% $HgCl_2$ for 15 minutes and after that these were then thoroughly rinsed 3-4 times with distilled water.

Preparation of seed beds

Six seed beds were prepared each of $1m^2$ area in two groups of 3 each in Botanical Garden, Gangasheel Mahavidyalaya, Faizullapur, Nawabganj, Bareilly. These seed beds were flooered by a polythene sheet at a depth of 40 cm to maintain constancy of ions. A mixture of soil and compost (3:1) was put in each seed bed. Seeds of susceptible variety were sown in one row and tolerant variety were sown in other row in the similar way.

Screening of varieties

Petriplate culture of Varshney and Baijal (1977) was followed for this purpose. The certified seeds of all ten varieties of field pea were allowed to germinate in petriplates under different solution NaCl (4, 8 $mScm^{-1}$). In each petriplate, 5 seeds were taken and the whole experiment was carried out in humidity controlled growth chamber at 27±1°C. Distilled water was used as control seed germination and root and shoot length were determined at an interval of 24 hours and continued upto 10 days.

Field Culture

The technique of Karadge and Chavan (1980) was adopted for field culture NaCl solution were added to the soil beds ($1m^2$) so as to fix the ECe of soil saturation extract at 4 and 8 $mScm^{-1}$. Ordinary garden soil served as control 1.2 $mScm^{-1}$). The seeds of two varieties of field pea *viz.* Pant pea-42 and Pant pea-66 were sown in beds in Botanical Garden, Gangasheel Mahavidyalaya, Faizullapur, Nawabganj, Bareilly. The treatments were applied weekly with properly watering the plants to avoid excessive salt accumulation and loss of H_2O due to evaporation from the soil.

Sampling

Plants from each plot were harvested randomly at three growth stage i.e. leafy stage (35 days after sowing) flowering stage (75 days after sowing) and maturity stage (160 days after sowing). The roots were dug out to their maximum length and adhering soil was removed off carefully by washing with tap water. Properly washed plants were separated into roots and shoots and blotted.

Nodulation

Number and size of nodules were recorded at leafy stage, flowering stage and maturity stage. Salinity affects the infection process by inhibiting root hair growth and decreasing the number of nodules per plant and the amount of nitrogen fixed per unit weight of nodules. Excess salt in the soil adversely affects survival, growth nodulation and legume – Rhizobium symbiosis (Rai 1992).

RESULTS AND DISCUSSION

Implementation of seeds

The different pea plant varieties taken into experiment, varied in their response to salinity. The influence of salinity except variety Pant pea-66 decreased negligible. In var. Pant pea-13, the germination percentage appreciated by 11.68% and 9.10% under 4 and 8 $mScm^{-1}$, (Table-1). In var. Pant pea-14, the germination percentage remained the same under 4 EC and appreciated by 7.31% under 8 $mScm^{-1}$. However, in var. Pant pea-25, the germination percentage decreased by 27.27% under treatment of 4 EC and increased under 8 $mScm^{-1}$ without exceeding control (Table-1). In var. Pant pea-42, the germination percentage appreciated by 13.25% and only 8.44% under 4 & 8 m Sem^{-1} respectively (Table-1). In var. Pant pea-66, the germination percentage appreciated by 6.29% under 4 EC and decreased marginally by 0.95% under 8 Scm^{-1}.

Root Length

A perusal of data given (Table-2) revealed that in general, the root length of field pea varieties increased with an expore of artificial salinization except var. Pant Pea-66 where the root length decreased under both 4 and 8 EC. In var. Pant Pea-13; Pant 14; Pant Pea-25; and Pant Pea-42, 4 EC level favoured the growth of root and 8 EC either maintained the growth.

The maximum increased root growth was recorded in var. Pant-13 under 4 m Scm^{-1} over control. Var. Pant Pea-66 reported itself as salinity susceptible since there was a gradual reduction in growth of root under 4 and 8 EC.

Shoot Length

The result given in (Table-2) that like root growth the shoot growth also increased with induction of artificial salinity except in var. Pant Pea-66, where it decreased gradually under increasing EC length (4-8 m Scm^{-1}). In var. Pant Pea-13, the shoot growth decreased by 18.03% over control under 4 EC and then increased by 39.34% over control under 8 EC. In var. Pant Pea-14, the shoot growth decreased by 14.28% over control under 4 EC and increased by 14.28% over control under 8 EC.

On the contrary in var. Pant Pea-25, the shoot growth increased by 2.58% over control under 4 EC and by 3.44% under 8 EC. In var. Pant Pea-42, the shoot growth increased by 41.53% over control under 4 EC and by

29.23% increased under 8 EC. In var. Pant Pea-66, the shoot growth decreased by 27.74% under 4 EC and 23.22% under 8 EC over control.

Thus the germination of seeds and early seedling growth of field pea varieties, var. Pant Pea-66 may be labelled as salinity susceptible and var. Pant Pea-42 as salinity tolerant.

Our results agree with those of Khajeh Hosseini *et al.,* 2003; Papadopoulos *et al*., 1985; Csizinsky, 1986; Coons and Pratt, 1988; Pessarakli and Tucker, 1988; Katerji *et al.,* 2001; who found significant differences in salt tolerance of different bean cultivars and several other pulse crops.

Our study illustrates the necessity of establishing and maintaining sources of genetic diversity for use in croip improvement and for countering present and future agriculture predicament.

Number and Size of nodules

Legumes growth in saline environments exhibit reduced number and size of root nodules (Table-3). The trend of decrement was recorded more or less identical in both the varieties of field pea. Variety Pant pea-66 was inherently superior nodulating variety. In var. Pant Pea-42 the plants at leafy stage showed 22.2% decrement in number of nodules over control with an increase in salinity levels. Plants of flowering stage showed 36.3% and 45.4% decrement in number of nodules in Var. Pant Pea-42 while correspondingly percentage in number of modules of Pant Pea-66 were recorded to be 25.0% and 41.6% over control under 4 and 8 Ece. Similarly, number of nodules in mature plants of Var. Pant pea-42 reduced by 33.3% and 50.3% and in var. Pant pea-66 by 23.0 and 53.8% over control under 4 and 8 ECe levels of salinity.

Plants of flowering stage showed 25.2% and 66.3% decrement over control in size of nodules in var. Pant pea-42 while correspondingly 31.6% and 70.4% over control under 4 and 8 8 ECe. Similarly, size of nodules in mature plants oif var. Pant pea-42 reduced by 26.0% and 71.5% and in var. Pant pea-66 by 29.0% and 70.2% over control under 4 and 8 ECe levels salinity.

Table.1: Germination of seeds of Field pea five varieties (*Pisum sativum* L.)

S.No.	Varieties	Control	EC 4	EC 8
1.	Pant Pea-13	64.16	71.66	70.00
2.	Pant Pea-14	68.33	68.33	73.33
3.	Pant Pea-25	73.33	53.33	73.33
4.	Pant Pea-42	69.16	78.33	75.00
5.	Pant Pea-66	68.99	75.33	68.33

Table 2 Effect of Salinity on root growth and shoot growth (cm) of five varieties of field pea. (*Pisum sativum* L.)

S.No.	Varieties	Root Growth			Shoot Growth		
		Control	EC4	EC8	Control	EC4	EC8
1.	Pant Pea-13	2.77	4.56	2.64	1.22	1.00	1.70
2.	Pant Pea-14	2.66	3.17	2.71	1.05	0.90	1.20
3.	Pant Pea-25	3.38	3.67	3.06	1.16	1.19	1.20
4.	Pant Pea-42	2.59	2.87	3.02	1.30	1.84	1.68
5.	Pant Pea-66	3.02	2.84	2.60	1.55	1.12	2.19

Table 3 Effect of Soil Salinity on nodulation in two varieties fo field pea (*Pisum satlivum* L). (values are mean of three replicates)

Growth	Parameters	Treatments Ece ($mSem^{-1}$)	Varieties	
			Pant Pea-42	Pant Pea-66
Leafy Stage	Size of Nodules	Control	0.76	0.81
		4	0.35	0.43
		8	0.21	0.24
	Number of nodules	Control	9	10
		4	7	8
		8	7	7
Flowering Stage	Size of Nodules	Control	0.95	0.98
		4	0.71	0.67
		8	0.32	0.29
	Number of nodules	Control	11	12
		4	7	9
		8	6	7
Maturity Stage	Size of Nodules	Control	1.23	1.31
		4	0.91	0.93
		8	0.35	0.39
	Number of nodules	Control	12	13
		4	8	10
		8	5	6

Leghaemoglobin contents

The plants of var. Pant pea 42 revealed percentage reduction in leghaemoglobin over control by 9.65%, 23.53% under 4 and 8 Ec at leafy stage; 7.69% and 38.49% under 4 ad 8 Ece at flowrig stage; and 12.14% and 49.76% under 4 and 8 Ece at maturity stage. Var. Pant Pea-66 showed correspondingly reduced percentage as 11.50% and 19.82% at leafy stage, 26.52% and 31.38 at flowering stage; and 1.10% and 19.42% at maturity stage under 4 and 8 Ece over control.

Our findings pertaining the Leghaemoglobin contents are in accordance with those of Delgao *et al.,* 1993; Camba *et al.,* 1998; Nandwal *et al.,* 2000; Swaraj *et al.,* 2000; Bouhmouch *et al.,* 2005; Bolanos *et al.,* 2006. Leghaemoglobin and active iron contents were suppressed by an increase in salinity.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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