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EFFECT OF PLANT GROWTH REGULATORS ON YIELD AND QUALITY OF BERSEEM (*TRIFOLIUM ALEXANDRINUM* L.) SEED

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SUMMARY

A field experiment was conducted at Forage Research Farm, Department of Plant Breeding & Genetics, Punjab Agricultural University, Ludhiana during **rabi** 2013-14 to study the effect of plant growth regulators viz., gibberellic acid 75 µg/ml - one spray, gibberellic acid 75 µg/ml–two sprays, cytokinin 50 µg/ml–one spray, cytokinin 50 µg/ml–two sprays, salicylic acid 75 µg/ml–one spray, salicylic acid 75 µg/ml–two sprays, naphthalene acetic acid 50 µg/ml–one spray, naphthalene acetic acid 50 µg/ml–two sprays, water spray and control on yield and quality of berseem seed. The application of one foliar spray of plant growth regulators was applied at flower initiation stage and second spray one week after the first spray. Among all the growth regulators, the application of one foliar spray of salicylic acid @ 75 µg/ml recorded significantly higher shoots/m^2, heads/m^2, seeds/head, 1000-seed weight, seed yield and seed quality i. e. germination per cent, root+shoot length and seedling vigour index and was also economical.

Key word : Berseem seed, gibberellic acid, cytokinin, salicylic acid, naphthalene acetic acid, seed yield, seed quality

Berseem (*Trifolium alexandrinum* L.) is one of the most important **rabi** legume fodder crops grown under irrigated conditions occupying about 2 million hectares in India. In Punjab, the area under this crop is more than 65 per cent of the total area under fodder crops and also known as milk multiplier. It remains soft and succulent at all stages of crop growth. It provides fodder over a longer period from November to May in 4-5 cuttings. The total fodder yield of this crop is comparatively more as compared to any other fodder crop of **rabi** season (Tiwana *et al.*, 2002). The seed productivity of berseem is generally low because farmers pay less attention to seed production causing serious seed shortages. Furthermore, clover seed production depends on the environmental conditions such as temperature and relative humidity, prevailing during the reproductive phase (Bakheit *et al.*, 2012). There is a shortage of good quality seed of berseem due to poor seed yield production under hot and dry conditions prevailing during flowering and seed setting. The seed yield of berseem could be enhanced through proper cutting management and application of growth regulators.The adoption of improved agronomic practices and plant growth regulators may also enhance the seed yield of berseem. Plant growth regulators other than nutrients, usually are organic compounds. They are either natural or synthetic compounds and are applied directly to a plant to alter its life processes or structure in some beneficial way so as to enhance yield, improve quality or facilitate harvesting. The use of bio-regulators is an emerging plant biotechnology approach which can modify plant gene expression, can affect levels of DNA, RNA, enzymes and finally their products such as protein, carbohydrates, lipids, allele-chemicals for enhancing yield and phyto-nutrients in food crops (Olaiya *et al.*, 2013). Growth rate of a plant is generally influenced by the environment but growth habit is determined largely by the interaction of internal factors including endogenous growth substances that control the activity of numerous meristems and thus influence the final form of the plant. Plant growth regulators (PGR's) are known to improve physiological efficiency including photosynthetic ability of plants. The PGR's are also known to enhance the source-sink relationship and stimulate the translocation of photo-assimilates. Abiotic stresses like heat stress in field crops can be managed by applying bio-regulators like salicylic acid, calcium chloride and sodium benzoate which are able to induce long term thermo-tolerance (Rab and Haq, 2012) and help to increase the flowering and pod formation in a number of crops including clovers. Salicylic acid retards ethylene synthesis, stimulates photosynthetic machinery, increases the chlorophyll content and is reported to increase the seed yield in cereals and legume under high temperature stress conditions (Hayat *et al.*, 2010). The foliar application of plant growth regulators at flower initiation stage is known to induce more flowering as well as seed setting and increase the seed production. Therefore, the present study was planned to study the effect of plant growth regulators on seed yield and quality of berseem.

MATERIALS AND METHODS

A field experiment was conducted during **rabi** 2013-14 at Forage Research Farm, Department of Plant Breeding & Genetics, Punjab Agricultural University, Ludhiana situated in Trans-Gangetic Agro-Climatic Zone and represents the Indo-Gangetic Alluvial Plains. It is located at 30.56^o N latitude, 75.52^o E longitude and at an altitude of 247 m above mean sea level. The climate of the area is characterized as sub-tropical and semi-arid with hot and dry spring-summer from April to June, hot and humid summer from July to September and cold autumn-winter from November to January. The average annual rainfall is about 705 mm, most of which is received during the monsoon period from July to September, while few showers are received during the winter season. The climate data were recorded at the meteorological observatory of Punjab Agricultural University, Ludhiana. The average maximum temperature ranged from 15.1 to 44.5^{o}C and average minimum temperature from 3.8 to 28.1^{o}C. A total of 160.2 mm rainfall was recorded during the crop growth season. Total weekly evaporation ranged from 4.6 to 85.2 mm. Mean daily sunshine hours ranged from 0.2 to 12.2 h. The overall weather during crop growth period remained favourable for berseem crop. The physical analysis indicated that soil of experimental field was loamy sand in texture. The chemical analysis of soil samples taken from 0-15 cm depth revealed that soil of the experimental field was normal in soil reaction (7.6) and electrical conductivity (0.18 dS/m), low in organic carbon (0.32%) and available nitrogen (253.9 kg/ha), medium in available phosphorus (21.1 kg/ha) and high in available potassium (332 kg/ha). The treatments included eight combinations of plant growth regulators such as gibberellic acid 75 µg/ml–one spray, gibberellic acid 75 µg/ml–two sprays, cytokinin 50 µg/ml–one spray, cytokinin 50 µg/ml–two sprays, salicylic acid 75 µg/ml–one spray, salicylic acid 75 µg/ml–two sprays, naphthalene acetic acid 50 µg/ml–one spray, naphthalene acetic acid 50 µg/ml–two sprays along with water spray and control. The application of one foliar spray of plant growth regulators was applied at flower initiation stage and second spray one week after the first spray. The berseem cultivar 'BL- 10' was sown with broadcasting method of sowing in the experiment and was laid out in a randomized complete block design with three replications. The crop was harvested for fodder yield and various growth parameters like plant height, shoots/m^2 and leaf : stem ratio were recorded at the time of harvesting in all the cuttings. The first cut was taken at 50 days after sowing and subsequent cuttings were harvested at 40 days intervals with sickle from the net plot area (4.50 x 3.75 m). The harvested green fodder was weighed plot-wise using hanging scale balance of 50 kg capacity and was converted to quintals per hectare. For quality analysis of fodder crop, samples were collected randomly from each plot at third cutting and were analyzed by the method described by AOAC (1990). For assessing the quality of seed produced, a sample of seed was collected and oven-dried to determine the moisture content of the seed. Germination percentage is the proportion of the number of seeds that are viable in a representative sample. A sample of 20 seeds collected within each plot was surface sterilized with 0.1 per cent mercuric chloride for 2 min and washed thoroughly. The seeds were placed in a petri-dish at equal distance and were covered with a lid to prevent the loss of moisture and were kept for germination at 25^{o}C in BOD incubator for seven days. The seeds germinated over a seven- day period were counted and expressed as germination per cent. Radical and hypocotyl lengths were measured seven days after the germination. Seedling vigour index (SVI) using the formula of Abdul-Baki and Anderson (1973) was as under :

$$\text{SVI} = \frac{\text{Seedling emergence percentage} \times \text{Total seedling length (mm)}}{100}$$

The expenditure incurred on tractor hours, irrigation, sprays, manuring, pesticides and bio-regulators in each treatment were worked out to obtain total cost of cultivation. However, the net profit was obtained after deducting the cost of cultivation from gross returns. In order to find out benefit : cost ratio, the net returns from individual treatment were divided by their respective cost of cultivation which included the cost of treatment also. The economics was calculated considering then prevailing prices of inputs and outputs. The selling rates of Rs. 75 per quintal for green fodder and Rs 10,000 per quintal for seed were taken as per the prevailing rates in the market.

RESULTS AND DISCUSSION

Fodder Yield and Quality

The berseem crop is a major fodder crop for feeding the livestock especially in late winters and summer season. The importance of any forage crop depends upon chemical composition and nutritive value which get influenced by variety of crops and climatic factors. All the growth and yield parameters increased up to third cut and reduced drastically in the fourth cut except leaf : stem ratio. Plant growth regulators did not show any effect on these parameters since no growth regulator was applied at this stage. The samples of third cut were analyzed to see the fodder quality because the crop was well established qualitatively as well as quantitatively. This crop is invariably rich in protein, ether extract, crude fibre, ash content, nitrogen free extract, calcium and phosphorus. Due to paucity of space, the cut-wise data are not given in Table 1. Singh (2008) also recorded the similar trend in green fodder yield, dry matter yield, plant height and shoots/m^2 in berseem crop at 50 days after sowing and the subsequent cuttings at 30-35 days intervals.

Seed Yield

All the growth regulators increased the seed yield and yield attributing characteristics over water spray and control significantly. The plant height with all the plant growth regulator treatments was significantly higher than water spray and control. But the difference between water spray and control was non-significant. The application of one spray of salicylic acid @ 75 µg/ml at the flower initiation stage recorded significantly higher plant height which was statistically at par with two sprays of salicylic acid @ 75 µg/ml and one spray of cytokinin @ 50 µg/ml. The maximum shoots per square metre, heads per square metre, number of seeds per head and 1000-seed weight were also recorded with the application of one spray of salicylic acid @ 75 µg/ml at the flower initiation stage. The highest seed yield of berseem was obtained with salicylic acid @ 75 µg/ml which was significantly higher than other growth regulator treatments and was followed by two sprays of salicylic acid @ 75 µg/ml (Table 2). Salicylic acid @ 75 µg/ml recorded 93.2 per cent higher seed yield over control and 21.8 to 75.6 per cent higher over rest of the growth regulator treatments. This increase in seed yield due to salicylic acid may be due to diversion of

TABLE 1
Fodder yield and quality of berseem

Treatment	Total green fodder yield (q/ha)	Total dry matter yield (q/ha)	Plant height (cm)	Shoots/ m^2	Leaf : stem	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash content (%)	Nitrogen free (%)
Gibberellic acid @ 75 µg/ml–one spray	763.7	78.4	34.6	296	1.26	17.4	2.52	24.5	13.4	42.2
Gibberellic acid @ 75 µg/ml–two sprays	766.9	76.5	34.2	294	1.27	17.8	2.75	25.3	14.2	40.0
Cytokinin @ 50 µg/ml–one spray	767.6	76.3	33.6	299	1.26	18.3	2.61	24.7	13.2	41.2
Cytokinin @ 50 µg/ml–two spray	771.5	77.3	33.9	295	1.18	18.1	2.65	25.4	13.6	40.3
Salicylic acid @ 75 µg/ml–one spray	766.5	78.3	35.4	306	1.33	17.6	2.62	24.5	14.3	41.0
Salicylic acid @ 75 µg/ml–two spray	766.7	79.1	33.2	297	1.37	17.3	2.83	25.5	13.7	40.7
Naphthalene acetic acid @ 50 µg/ml–one spray	766.6	75.6	32.7	300	1.23	18.2	2.58	24.3	13.3	41.6
Naphthalene acetic acid @ 50 µg/ml–two spray	765.9	76.3	33.7	299	1.34	17.9	2.55	25.2	14.4	40.0
Water spray	765.8	76.3	34.4	293	1.25	18.2	2.56	24.2	13.1	41.9
Control	764.5	78.2	32.9	287	1.11	18.1	2.53	25.3	13.5	40.6
S. Em	24.6	2.33	0.60	11.6	0.02	0.54	0.13	0.67	0.52	0.97
C. D. (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS–Not Significant.

TABLE 2
Effect of plant growth regulators on yield attributing characteristics and seed yield of berseem

Treatment	Plant height (cm)	Shoots/m^2	Heads/m^2	Seeds/ head	1000-seed weight (g)	Seed yield (q/ha)	Straw yield (q/ha)	Harvest index
Gibberellic acid @ 75 µg/ml–one spray	49.1	439.5	426.5	46.3	2.44	7.58	41.1	15.3
Gibberellic acid @ 75 µg/ml–two spray	50.1	440.7	428.7	46.7	2.55	7.90	41.3	16.0
Cytokinin @ 50 µg/ml–one spray	53.6	459.5	443.5	48.9	2.83	8.77	44.0	16.7
Cytokinin @ 50 µg/ml–two sprays	51.6	455.6	446.6	47.2	2.46	8.17	42.7	16.3
Salicylic acid @ 75 µg/ml–one spray	54.5	477.7	471.7	53.4	2.91	10.7	49.5	18.0
Salicylic acid @ 75 µg/ml–two sprays	53.6	474.4	469.4	50.5	2.73	10.3	49.2	17.3
Naphthalene acetic acid @ 50 µg/ml–one spray	47.8	453.3	444.7	45.8	2.54	7.48	40.7	15.3
Naphthalene acetic acid @ 50 µg/ml–two sprays	50.4	455.5	444.5	46.6	2.64	8.59	43.6	16.7
Water spray	46.7	425.9	421.2	44.0	2.34	6.08	38.0	13.7
Control	46.4	421.2	418.2	40.3	2.23	5.53	35.9	13.3
S. Em	1.44	11.4	11.7	1.32	0.07	0.38	1.49	0.59
C. D. (P=0.05)	4.28	33.9	34.8	3.90	0.2	1.15	4.4	1.8

photosynthates from vegetative parts to the reproductive parts and may also be due to higher number of shoots/ m^2, heads/m^2 and seeds/head. Kumar *et al*. (2013) also reported highest seed yield with the foliar spray of salicylic acid @ 50 mg/l at flower initiation stage. The increased seed yield with foliar application of salicylic acid @ 50 mg/l was due to higher number of heads/m^2, seeds/head and 1000-seed weight. Kumar *et al*. (2014) also reported 32.7 per cent increase in seed yield of forage cowpea over control with the application of 50 µg/ml salicylic acid. The application of one spray of salicylic acid @ 75 µg/ml at flower initiation also recorded highest straw yield and harvest index.

Seed Quality

The main criteria for describing seed quality included seed germination and seedling vigour. Quality seed ensures good germination, rapid emergence, vigorous growth and good crop stand in the field. All the plant growth regulators did not show significant effect on germination percentage and moisture content of berseem seed but significantly influenced the root+shoot length and seedling vigour index. Salicylic acid @ 75 µg/ml resulted in maximum root+shoot length and seedling vigour index (Table 3). The protective and growth promoting effects of salicylic acid led to production of healthy seeds which resulted in better seedling growth. Gutierrez-Coronado *et al*. (1998) also reported that foliar application of salicylic acid significantly increased the growth of shoots and roots of soybean both in greenhouse and field condition.

TABLE 3
ffect of plant growth regulators on seed quality in berseem seed

Treatment	Moisture content (%)	Germination (%)	Root+shoot length (cm)	Seedling vigour index
Gibberellic acid @ 75 µg/ml–one spray	11.7	86.9	7.2	62.7
Gibberellic acid @ 75 µg/ml–two sprays	11.8	88.1	7.4	65.1
Cytokinin @ 50 µg/ml–one spray	12.3	91.2	7.6	70.8
Cytokinin @ 50 µg/ml–two sprays	12.2	90.6	7.5	68.2
Salicylic acid @ 75 µg/ml–one spray	13.2	93.5	8.1	75.2
Salicylic acid @ 75 µg/ml–two sprays	12.3	92.8	7.7	70.8
Naphthalene acetic acid @ 50 µg/ml–one spray	11.6	86.7	7.1	61.5
Naphthalene acetic acid @ 50 µg/ml–two sprays	11.8	88.7	7.3	65.2
Water spray	10.7	84.8	6.9	58.8
Control	10.6	83.0	6.3	52.7
S. Em	0.66	4.77	0.29	17.6
C. D. (P=0.05)	NS	NS	0.88	5.23

TABLE 4
Effect of plant growth regulators on economics of berseem

Treatment	Gross returns (Rs./ha)	Total cost (Rs./ha)	Net returns (Rs./ha)	B : C ratio
Gibberellic acid @ 75 µg/ml–one spray	133278	81153	52125	0.64
Gibberellic acid @ 75 µg/ml–two spray	136518	85753	50765	0.59
Cytokinin @ 50 µg/ml–one spray	145570	85750	59820	0.70
Cytokinin @ 50 µg/ml–two spray	139863	94948	44915	0.47
Salicylic acid @ 75 µg/ml–one spray	164488	76598	87890	1.15
Salicylic acid @ 75 µg/ml–two spray	160503	76640	83863	1.09
Naphthalene acetic acid @ 50 µg/ml–one spray	132495	76738	55757	0.73
Naphthalene acetic acid @ 50 µg/ml–two spray	143443	76925	66518	0.86
Water spray	118435	76553	41882	0.55
Control	112338	75873	36465	0.48

Economic Evaluation

Economic analysis of a crop decides the economic viability of the applied treatment and it also provides the benefit : cost ratio of the applied treatments. The data presented in Table 4 reveal that one spray of salicylic acid recorded significantly higher gross returns, net returns and benefit : cost ratio over rest of the treatments. This treatment recorded Rs. 164488 per hectare gross returns and Rs. 87890 per hectare net returns and benefit : cost ratio of 1.15 and these were highest among all the treatments. This might be due to higher seed yield of berseem with one spray of salicylic acid @ 75 µg/ml. The higher net returns and benefit : cost ratio from the forage cowpea was also recorded by Kumar *et al*. (2014) when salicylic acid @ 50 µg/ml were sprayed on crop at flower initiation stage.

Based on the above mentioned findings, it may be concluded that the foliar application of salicylic acid @ 75 µg/ml at flower initiation stage was found to be most effective in increasing the growth and yield attributes as well as seed yield in berseem and was also economically viable.

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