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Enhancement of seed setting in Lucerne through foliar spray of nutrients and growth regulators

Murali Bellamkonda, Shanti Madaraju, Shailaja Kola, Shashikala Tummanepally and Susheela Ravipati

Abstract
Lucerne is an important legume fodder crop that supplies nutritious and palatable fodder for livestock. Green fodder supply is a problem in the *rabi* and summer seasons in India. Lucerne can be a better option for addressing this problem. Hence many farmers showing interest in growing Lucerne. However, the availability of sufficient Quantiles of quality seed is a big problem in Lucerne due to its poor seed setting nature. Nutrients and plant growth regulators play a vital role in improving seed setting for enhancing seed yield in many crops is well studied. With this background, field experiments were carried out in rabi 2017-118 and 2018-19 at Agricultural Research Institute Prof. Jayashankar Telangana State Agricultural University Rajendranagar Hyderabad. Eight treatments, consisting of three nutrients and four growth regulators, and control tested in an RCBD with three replications. Two-year pooled analysis results revealed that foliar application of brassinolide @ 100 ppm twice, one at 50% flowering and the second at 15 days after the first recorded highest seed yield of 201.1 kg/ha, with a net return of Rs. 78,959/ha, and BC ratio of 1:2.22 followed by mepiquat chloride @ 500 ppm sprayed twice with 186.5 kg/ha, Rs. 68,239 and 1:1.97 seed yield, net returns and B:C ratio respectively compared to the other treatments *viz*., ZnSO4 @ 0.25%, K2SO4 @1.0%, MAP @1.0%, Salicylic acid @100 ppm, TNAU pulse wonder @ 1.0%, and control.

Keywords: Lucerne, foliar spray, nutrients, growth regulators, seed setting, seed yield

1. Introduction
Lucerne (*Medicago sativa* L.) is the Queen of forage crops, the most widespread forage legume in the world. Lucerne is also called alfalfa in most parts of the world. Lucerne is a native of the temperate region; hence it can tolerate low temperatures well. But it is also cultivated in many tropical countries successfully. Lucerne grows in one million ha in India with a total green fodder production of 60-130 tons per hectare per annum. Lucerne occupies the third position among fodder crops after sorghum and berseem in India. Lucerne is generally grown during the rabi season as an irrigated crop commonly in Punjab, Haryana, Uttar Pradesh, Rajasthan, Gujarat, Maharashtra, and Tamilnadu in India. With its deeper root system, it is well suited to dry areas even with inadequate irrigation facilities. In high water table areas, it can grow well as a rainfed crop. It is a persistent, productive, and drought-tolerant fodder legume. It has 15% crude protein and 72% digestible dry matter (Kumar *et al.*, 2012) [1], and it is estimated to fix 83-594 kg nitrogen per ha per annum. It can supply green fodder for a prolonged period (November to June) than Berseem (December to Aril) in India. With these good qualities, livestock farmers are interested in growing Lucerne in many parts of India, including non-traditional Lucerne growing regions. However, the availability of sufficient quantities of quality seed is an issue because the main problem with Lucerne is its low seed-producing capacity due to its poor seed setting nature. The poor seed setting is because of less translocation of water-soluble sugars from vegetative to reproductive organs, the low productive potential of flowers developed in the later stage. Other reasons for low seed yield in Lucerne are non-synchronous flowering behavior, prolonged flower drop, uneven pod maturity, and pod shattering. Hence, seed yield in Lucerne crop is commonly ranges between 2.0 to 4.5 q/ha.

Many studies revealed that seed setting in Lucerne is affected by nutrition and plant growth regulators. The agronomic factors that affect seed production in Lucerne are sowing time, soil fertility, cutting management, insect pollination, conducive climatic conditions, and seed maturity at harvest. Commercial varieties of Lucerne are completely self-sterile; so seed production requires pollinators when the fields of Lucerne are in bloom.

Plant growth substance are known to enhance the source – sink relationship and stimulate the translocation of photo-assimilates there by helping in effective flower formation, fruit and seed development and ultimately enhance the productivity of crops (Toungos. 2018) [2]. Ma and Smith (1992) [3] observed that "PGR application does not increase total dry matter accumulation on a per-shoot basis, but does affect dry matter partitioning patterns, leading to alterations in grain yield that may be positive or negative depending on the conditions during a given crop year" in spring barley. Zinc had no positive effect on seed yield and yield components, whereas molybdenum and boron had a seed yield increase of 27 - 47% and 22 - 35% respectively. Among seed yield components, the number of pods per raceme was the most important factor to determine alfalfa seed yield (Du *et al.*, 2009) [4]. Khrbeet (2018) [5] reported that the highest seed yield was obtained with the foliar application of zinc at the concentration of 60 mg per litre on local variety. Foliar application of 2% KCl at 50% flowering and also at 50% podding stages of lentil crop was found to be effective in maintaining the higher the seed yield under rice-fallow condition (Yadav *et al.*, 2021) [6]. Sivakumar *et al.* (2019) [7] reported that foliar application of 0.5% mono-ammonium phosphate (MAP) twice; first spray at flowering and the second spray at 15 days after first spray obtained significantly higher seed yield in redgram than the similar application of 2% DAP and foliar application of TNAU pulse-wonder at 5 kg/ha at peak flowering. Salicylic acid plays exclusive role in plant growth, thermogenesis, flower induction and uptake of ions. It affects ethylene biosynthesis, stomatal movement and also reverses the effects of ABA on leaf abscission (Yusuf *et al.* 2013) [8]. The plant height, panicle length, panicles number, grain weight per panicle, seed index and grain protein content as well as biomass, grain, and protein yields were significantly increased by salicylic acid application in pearl millet (Salem 2020) [9]. Foliar application of salicylic acid at 50 mg L^{-1} and KNO_3 at 2% recorded the maximum seed yield and seed quality (Kumar *et al.*, 2013) [10]. Wassie *et al.* (2013) [11] found that exogenous SA application enhanced alfalfa heat tolerance by modulating various morphological and physiological characteristics under heat stress significantly at concentration of 0.25 mM. Chen *et al.* (2016) [12] recorded higher seed yield in alfalfa with a foliar application of chlormequat chloride (CCC) at the stooling stage and two foliar sprays of NAA; one at flower bud initiation and the second one at peak flowering. Mepiquat chloride application increased lint yield in cotton and useful for reducing plant height regardless of population (Siebert and Stewart 2006) [13]. Plant height, number of branches per plant, Leaf Area Index, dry matter production, and grain yield was significantly higher with the foliar application of TNAU pulse wonder @ 2 kg per acre at 15, 30, 45 and 60 days after sowing in greengram (Sridhar *et al.*, 2020) [14]. Kavitha *et al.* (2019) [15] reported that application of 125% RDF along with 2% DAP spray at flowering and pod formation stages recorded higher pod yield followed by 125% RDF + Pulse wonder @ 5 kg ha^{-1} at flowering in vegetable cowpea. Sridhara *et al.* (2021) [16] recorded that application of homobrassinolide @ 0.12 a.i. per ha increased number fruits per plant, fruit length, fruit weight, total fruit yield, and fruit quality parameters such as fruit firmness, ascorbic acid content, and TSS in tomato. Sairam (1994) [17] found that homobrassinolide application in wheat resulted in increased relative water content, nitrate reductase activity, chlorophyll content and photosynthesis under irrigated and water-stress conditions. He had recorded higher leaf area, biomass production, grain yield and yield related parameters in the treated plants. With this background the present study was taken up to identify a suitable nutrient or plant growth regulator for enhancing seed setting and increasing seed yield of Lucerne in rabi season of 2017-18 and 2018-19 at Agricultural Research Institute, Prof. Jayashnakar Telangana State Agricultural University Rajendranagar Hyderabad.

2. Materials and Methods

A field trial was conducted during rabi 2017-18 and repeated in rabi 2018-19. The experimental location is situated at 17°19' 18" N latitude, 78°24' 18" E longitude and at an altitude of 527m above mean sea level in the Southern Telangana Agroclimatic Zone in Telangana State India.The average annual rainfall of the area was 750 mm and maximum and minimum temperatures ranged between 24.6 to 34.1^{0}C and7.6 to 18.6^{0}C respectively during the crop growth period. Experimental site was well drained moderately deep sandy loam soil with pH of 7.8 and EC of 0.22 dS m-1. The experimental field was low in available N (152 kg ha^{-1}), medium in phosphorus (26.0 kg ha^{-1}) and high in potash (293.0kg ha^{-1}). The experiment was conducted with eight treatments using a randomized complete block design with three replications. Recommended doses of nitrogen, phosphorus, and potassium were applied in the form of urea, single super phosphate, and muriate of potash respectively. Full dose of SSP and MOP were applied to the experimental plots as basal dose and the total quantity of urea was applied in 4 equal split applications including a basal application. The remaining three splits were applied at 30-day intervals. RL 88 variety of Lucerne was sown in the last week of November both the years (2017 & 2018) at a spacing of 30 cm wide solid rows. Initial vegetative growth of the crop has been harvested in the second fortnight of January to encourage branching of the crop and left the crop for seed production purposes. The crop was evaluated for number of days taken to attain maturity, number of pods per plant, number of seeds per pod, fertility ratio (%), 1000 seed weight (g) and seed yield. Fertility ratio is the ratio between filled seeds to unfilled seeds in a pod and expressed in % by multiplying the ratio with 100. The collected data was statistically analysed by analysis of variance (ANOVA) for randomised complete block design. Critical differences were worked out at five percent probability level in LSD, if treatments were significantly differed and if not; NS was denoted (Gomez and Gomez 1984) [18]. Two years data was pooled and statistically analysed using OPSTAT software for the interpretations of results (Sheron *et al.*, 1998) [19].

Fig 1: Field view of Lucerne experiment at ARI Rajendranagar, Hyderabad

3. Results and Discussion

3.1 Number of days for maturity

The two-year pooled data analysis results have shown that they differed significantly (at 5%) in number of days taken to maturity. Foliar application of MAP @ 1% twice one at 50% flowering and second one at 15 days after the first spray had given early maturity (172 days from sowing) followed by mepiquat chloride @ 500 ppm sprayed on foliage twice in similar way attained maturity in 172.8 days (Table 1). These are significantly earlier than other treatments. Perhaps phosphorus in MAP has given the effect of early maturity and the growth regulator mepiquat chloride generally reduces vegetative growth that might have stimulated the early reproductive phase led to early maturity, similar results reported by Siebert and Stewart, (2006) [13] in cotton. In control treatment (no foliar spray) maturity was attained in 179 days from sowing date.

3.2 Number of pods per plant

The results from the two-year pooled analysis revealed that treatments significantly differed in producing number of pods per plant (Table 1). The highest number of pods per plant (33.2) was recorded with brassinolide @ 100 ppm treatment, followed by mepiquat choloride @ 500 ppm (31.4) and MAP @1.0% (31.2). A result which agrees with that of Sairam (1994) [17] who reported that increase of yield attributes in wheat when applied as foliar spray of homobrassinolide on foliage; foliar application of chlormequat @ 0.3% on Lucerne increased the number of pods per raceme by 178% (Du *et al.*, 2008) [4]. Brassinolide regulates positively in increasing the number pods per plant by regulating the photosynthetic efficiency and carbohydrate translocation to yield components.

3.3 Number of seeds per pod

The highest number of seeds per pod, 8.4, was recorded with the foliar spray of brassinolide @ 100 ppm. Which was significantly higher than other treatments except mepiquat chloride sprayed @ 500 ppm recorded 8.0 seeds per pod (Table 1). Foliar application of MAP @ 1.0% recorded 7.5 seeds per pod and $ZnSO_4$ @ 0.25% recorded 7.4 seeds per pod. These two treatments were also significantly higher than the control (6.6 seeds/pod). Similar results of increased yield attributes in wheat reported by Sairam (1994) [17].

3.4 Fertility ratio: The two-year pooled data analysis results (Table 1) have shown that higher fertility ratio was recorded with the foliar spray of brassinolide @ 100 ppm (112.6%) followed by foliar application of MAP @ 1.0% (108.8%) and mepiquat chloride @ 500 ppm (108.7%). Foliar spray of nutrients and growth regulators might have influenced the ratio of filled seed number to unfilled seed number in pods; that leads to an increase of fertility ratio.

3.5 Thousand (1000) seed weight (gr)

The highest 1000 seed weight recorded was 3.22 g with brassinolide @ 100 ppm foliar spray, followed by foliar application of K_2SO_4 @ 1.0%, 3.16 g and MAP @ 1.0% 3.10 g. However, 1000 seed weight did not differ significantly between treatments in Lucerne (Table 1).

3.6 Seed Yield (Kg/ha)

The results from the two-year pooled analysis revealed that treatments significantly differed in the production of seed yield (Table 1). Brassinolide @ 100 ppm recorded the highest seed yield, 201.1 kg/ha followed by mepiquat chloride @ 500 ppm with 186.5 kg/ha and 179.9 kg/ ha seed yield compared to the other treatments *viz.*, $ZnSO_4$ @ 0.25%, K_2SO_4 @ 1.0%, MAP @1.0%, Salicylic acid @100 ppm, TNAU pulse wonder @ 1.0%, and control (no spray) which varied from 149.5 to 173.0 kg seed yield per ha. Similar results of increased yield have been reported by Sairam (1994) [13] in wheat by foliar spraying foliar of homobrassinolide and (Sridhara *et al.*, 2021) [16] in tomatoes with foliar spray of homobrassinolide @ 0.04%. The year 2018-19 has been found better in producing higher seed yield attributing parameters and seed yield/ha than the year 2017-18 (Appendix 1), which may be due to the favorable environment that occurred in the year 2018-19.

3.7 Economics of seed production

Treatments significantly differed in seed production economic parameters such as net returns and BC ratio (Table 2). Two foliar sprays of brassinolide @ 100 ppm, one at 50% flowering time and the second 15 days after the first spray, recorded a net return of Rs. 78,959/ha and BC ratio of 1: 2.22 with producing highest seed yield of 201 kg/ha followed by mepiquat chloride @ 500 ppm with a net return of Rs. 68,239 and 1:1.97 BC ratio. These two were significantly higher compared to the other treatments *viz.*, $ZnSO_4$ @ 0.25%, K_2SO_4 @1.0%, MAP @ 1.0%, Salicylic acid @ 100 ppm, TNAU pulse wonder @ 1.0%, and control (no foliar spray).

Table 1: Influence of foliar spray of nutrients & PGRs on Lucerne yield attributes and seed yield

Treatments	No. of Days for Maturity	No. of Pods per Plant	No. of Seeds per pod	Fertility ratio (%)	1000 seed weight (g)	Seed Yield (Kg/Ha)
T1: $ZnSO_4$ @ 0.25%	174.1	29.6	7.4	106.4	2.99	173.0
T2: K_2SO_4 @ 1.0%	176.5	27.8	7.0	103.6	3.16	172.4
T3: MAP @ 1.0%	172.0	31.2	7.5	108.8	3.10	179.7
T4: Salicylic Acid @ 100 ppm	175.1	29.0	7.1	105.6	3.03	163.8
T5: Mepiquat Chloride @ 500 ppm	172.8	31.4	8.0	108.7	3.05	186.5
T6: TNAU Pulse wonder @ 1.0%	175.5	28.1	7.1	104.2	2.97	164.9
T7: Brassinolide @ 100ppm	177.0	33.2	8.4	112.6	3.22	201.1
T8: Control (No foliar spray)	179.0	25.4	6.6	100.7	2.92	149.5
C.D. (P=0.05)	1.9***	2.4***	0.8**	6.6	0.09**	24.1**
SE(m)	0.6	0.8	0.2	2.3	0.03	8.4

Note: Values presented in the table are two-year (2017-18 & 2018-19) pooled analysis results only and year-wise including pooled data tables were presented in appendix.

Table 2: Influence of foliar spray of nutrients &PGRs on economics of Lucerne seed cultivation

Treatments	Cost of Cultivation (Rs/Ha)	Gross Returns (Rs/Ha)	Net Returns (Rs/Ha)	BC Ratio
T1: $ZnSO_4$ @ 0.25%	61,012	1,17,813	66,053	1.84
T2: K_2SO_4 @ 1.0%	60,482	1,12,082	63,313	1.85
T3: MAP @ 1.0%	60,708	1,16,822	65,818	1.92
T4: Salicylic Acid @ 100 ppm	61,243	1,06,525	58,763	1.74
T5: Mepiquat Chloride @ 500 ppm	61,375	1,21,262	70,238	1.97
T6: TNAU Pulse wonder @ 1.0%	61,183	1,07,188	60,235	1.75
T7: Brassinolide @ 100ppm	61,220	1,36,163	78,960	2.22
T8: Control (No foliar spray)	60,258	97,237	52,623	1.61
C.D. (P=0.05)	630**	10823***	12720**	0.22***
SE(m)	219	3769	4430	0.07

Note: Values presented in the table are two-year (2017-18 & 2018-19) pooled analysis results only and year-wise including pooled data tables were presented in appendix.

4. Conclusions
Foliar application of brassinolide @ 100 ppm twice, one at 50% flowering and the second at 15 days after the first recorded highest seed yield of 201.1 kg/ha, with a net return of Rs. 78,959/ha, and BC ratio of 1: 2.22 followed by mepiquat chloride @ 500 ppm sprayed twice with 186.5 kg/ha, Rs. 68,239 and 1:1.97 seed yield, net returns and B:C ratio respectively compared to the other treatments *viz*., ZnSO4 @ 0.25%, K2SO4 @1.0%, MAP @1.0%, Salicylic acid @100 ppm, TNAU pulse wonder @ 1.0%, and control (no spray). Therefore, to improve seed setting and increase seed yield in Lucerne, spraying brassinolide @ 100ppm twice, one at 50% flowering time and the second at 15 days after the first spray, is recommended.

5. Appendix: Results of the year 2017-18, 2018-19, and 2 years pooled data was given in two tables.

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Appendix 1

Table 1: Influence of foliar spray of nutrients & PGRs on Lucerne yield attributes and seed yield

	Number of days for maturity			Number of pods per plant			Number of seeds per pod			Fertility ratio			1000 seed weight (gr)			Seed Yield (Kg/ha)		
Treatments	**2017-18**	**2018-19**	**2 years Pooled**	**2017-18**	**2018-19**	**2 years Pooled**	**2017-18**	**2018-19**	**2 years Pooled**	**2017-18**	**2018-19**	**2 years Pooled**	**2017-18**	**2018-19**	**2 years Pooled**	**2017-18**	**2018-19**	**2 years Pooled**
T1:ZnSO4 @ 0.25%	173	175	174.1	24.4	35.0	29.6	5.9	9.0	7.4	105.8	107.0	106.4	2.95	3.04	2.99	168.79	182.20	173.00
T2:K2SO4 @ 1.0%	174	179	176.5	23.7	32.0	27.8	5.6	8.5	7.0	102.3	105.0	103.6	3.14	3.19	3.16	167.36	177.50	172.40
T3:MAP @ 1.0%	172	172	172.0	27.7	36.0	31.2	6.1	9.2	7.5	108.6	109.0	108.8	3.13	3.08	3.10	172.35	190.00	179.70
T4:Salicylic Acid @100ppm	174	176	175.1	25.0	34.0	29.0	5.8	8.6	7.1	104.3	107.0	105.6	2.90	3.16	3.03	153.72	172.21	163.80
T5:Mepiquat Chloride @ 500 ppm	172	173	172.8	25.2	37.0	31.4	6.2	9.9	8.0	107.4	110.0	108.7	3.04	3.06	3.05	175.13	197.41	186.50
T6:TNAU Pulse wonder @ 1.0%	175	176	175.5	23.2	33.0	28.1	5.7	8.7	7.1	102.5	106.0	104.2	2.96	2.98	2.97	158.19	170.78	164.90
T7:Brassinolide @ 100ppm	176	178	177.0	27.6	39.0	33.2	6.9	10.1	8.4	111.5	114.0	112.6	3.10	3.34	3.22	197.36	218.26	201.10
T8:Control (No foliar spray)	177	181	179.0	20.9	30.0	25.4	5.2	8.2	6.6	100.5	101.0	100.7	2.93	2.92	2.92	139.58	159.60	149.50
C.D. (Treat) P=0.05	1.8	3.4	1.9***	NS	2.2	2.4***	0.4	1.5	0.8**	NS	1.8	6.6	0.17	0.12	0.09**	18.5	4.9	24.1**
SE(m)(Treat)	0.6	1.1	0.6	1.4	0.7	0.8	0.1	0.4	0.2	5	0.6	2.3	0.05	0.04	0.03	6.0	1.6	8.4
CD (Envi)P=0.05			0.9***			1.2***			0.4***			3.3			0.04***			12.0**
SE(m)(Envi)			0.3			0.4			0.1			1.1			0.01			4.2

Table 2: Influence of foliar spray of nutrients & PGRs on economics of Lucerne seed cultivation

	Cost of Cultivation (Rs/ha)			Gross Returns (Rs/ha)			Net Returns (Rs/ha)			BC ratio		
Treatments	**2017-18**	**2018-19**	**2 years Pooled**	**2017-18**	**2018-19**	**2 years Pooled**	**2017-18**	**2018-19**	**2 years Pooled**	**2017-18**	**2018-19**	**2 years Pooled**
T1:ZnSO4 @ 0.25%	60,943	61,080	61,012	1,09,714	1,18,430	1,17,813	48,771	57,350	66,053	1.8	1.94	1.84
T2:K2SO4 @ 1.0%	60,541	60,420	60,482	1,08,785	1,15,375	1,12,082	48,243	54,955	63,313	1.79	1.9	1.85
T3: MAP @ 1.0%	60,616	60,800	60,708	1,12,027	1,23,500	1,16,822	51,410	62,700	65,818	1.84	2.03	1.92
T4: Salicylic Acid @100ppm	60,986	61,500	61,243	99,921	1,11,941	1,06,525	38,935	50,441	58,763	1.63	1.82	1.74
T5: Mepiquat Chloride @ 500 ppm	61,350	61,400	61,375	1,13,836	1,28,321	1,21,262	52,486	66,921	70,238	1.85	2.09	1.97
T6: TNAU Pulse wonder @ 1.0%	61,016	61,350	61,183	1,02,826	1,11,009	1,07,188	41,810	49,659	60,235	1.68	1.81	1.75
T7: Brassinolide @100ppm	60,990	61,450	61,220	1,28,285	1,41,873	1,36,163	67,295	80,423	78,960	2.1	2.31	2.22
T8: Control (No foliar spray)	60,416	60,100	60,258	90,729	1,03,740	97,237	30,313	43,640	52,623	1.5	1.72	1.61
C.D. (Treat) P=0.05	NS	NS	630**	12,051	3,234	10823***	12,039	3,641	12720**	0.19	0.07	0.22***
SE(m)(Treat)	237	405.6	219	3,935	1,056	3769	3,931	1,189	4430	0.06	0.02	0.07
CD (Envi) P=0.05			315			5411**			6360***			0.11**
SE(m)(Envi)			109			1884			2215			0.03