Gibberellic acid mediated co-ordination of calcium and magnesium ameliorate physiological activities, seed yield and fibre yield of *Linum usitatissimum* L.—a dual-purpose crop

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Abstract Commercial cultivation of linseed for both seed and fibre is not keeping pace with increasing demand for linseed products. Although, different strategies are being adopted to produce a dual-purpose linseed crop with good yield of seed and fibre, little progress has been achieved. The present study was carried out to investigate whether application of gibberellic acid (GA_3) along with $CaCl_2$ and/or $MgSO_4$ could ameliorate the seed yield in three linseed genotypes ‘Parvati’, ‘Shekhar’ and ‘Shubhra’ without compromising the fibre production. Before sowing the seeds of linseed genotypes were soaked for 8 h in 10^{-6} M GA_3 . Forty days after sowing (DAS), the plants were sprayed with 10^{-6} M GA_3 along with 2 kg Ca/ha (Ca_2) and/or 0.5 kg Mg/ha ($Mg_{0.5}$). Treatments comprised of (1) 10^{-6} M GA_3 + Ca_0Mg_0 (control, T0); (2) 10^{-6} M GA_3 + Ca_2Mg_0 (T1); (3) 10^{-6} M GA_3 + $Ca_0Mg_{0.5}$ (T2) and (4) 10^{-6} M GA_3 + $Ca_2Mg_{0.5}$ (T3). Performance of the crop was assessed in terms of growth characteristics, physiological and biochemical parameters at 60 and 75 DAS and yield and quality attributes at harvest. Treatment T3 proved best, it enhanced dry weight per plant by 38.2 and 20.6%, P_N by 20.7 and 19.1% and gs by 18.2 and 8.8% at 60 and 75 DAS, respectively and seed yield by 39.6%, oil yield by 46.9% and fibre yield by 36.9% at harvest. Further, a decrease in lodging by 13.9% was recorded. Of the three

genotypes tested, all exhibited significant difference for all the parameters studied, except for leaf-N content, biological yield and iodine value which showed no difference. However, ‘Shubhra’ performed better than ‘Parvati’.

Keywords Linseed · Fibre yield · Lodging · Photosynthesis · Carbonic anhydrase

Introduction

Seeds of linseed (*Linum usitatissimum* L.), an important industrial crop, are used for the extraction of oil and stem for the fibres. About 80% of linseed oil is used in industry and the remaining for edible purpose (Verma et al. 2005). The stem fibre is used in the manufacture of canvas, coating, durries, shirting and strong twines. The good quality fibre is used for linen. The woody portion of stem left after the removal of fibre, is a source of paper manufacture (Samba Murty and Subrahmanyam 1989).

Commercial cultivation of linseed is clearly not cost effective and is not able to keep pace with increasing demands of linseed products. One of the best strategies for dual-purpose linseed would be to increase the height of the plant and to improve seed weight. To achieve this, plant growth regulators have been used as they are known to affect many facets of plant life, including growth, flowering, fruiting and ion transport (Khan and Samiullah 2003; Siddiqui et al. 2008). It is well established that gibberellins promote growth through cell expansion by stimulating the destruction of growth-repressing proteins (Achard et al. 2009). Ca is involved in cell division, cell elongation and tissue extensibility (Hirschi 2004) and Mg is the constituent of chlorophyll molecule. It acts as a bridging element for the aggregation of

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ribosomes; plays a key role in photosynthesis and is essential for the activity of many enzymes involved in photosynthesis (Mengel and Kirkby 1996; Cakmak and Kirkby 2008).

In the present study it was planned to apply GA₃ to linseed to increase stem height. Application of calcium chloride and magnesium sulphate was to provide mechanical support to the plants by strengthening the middle lamellae.

Materials and methods

Soil analysis

Prior to seed sowing, homogenous mixture of soil and farmyard manure (3:1) were analysed for physico-chemical properties. The soil consisted of a texture-sandy loam, pH (1:2) 7.8, E.C. (1:2) 0.65 m dS/m, available N (97.05), P (7.90) and K (157.02) mg per kg soil, and 0.11% calcium carbonate.

Plant material and experimental design

A factorial randomized pot experiment was carried out at the Department of Botany, Aligarh Muslim University, Aligarh, (27° 52' N latitude, 78° 51' E longitude and 187.45 m altitude), India. Healthy seeds of linseed genotypes, 'Parvati', 'Shekhar' and 'Shubhra' were obtained from the Division of Oilseed Crops of the Chandra Shekhar Azad University of Agriculture and Technology, Kanpur (Uttar Pradesh). Before sowing, the seeds were soaked for 8 h in 10⁻⁶ M GA₃ solution. Forty days after sowing (DAS), the plants were sprayed with 10⁻⁶ M GA₃ along with 2 kg Ca/ha (Ca₂) and/or 0.5 kg Mg/ha (Mg_{0.5}). Treatments comprised of (1) 10⁻⁶ M GA₃ + Ca₀Mg₀ (control, T0); (2) 10⁻⁶ M GA₃ + Ca₂Mg₀ (T1); (3) 10⁻⁶ M GA₃ + Ca₀Mg_{0.5} (T2) and (4) 10⁻⁶ M GA₃ + Ca₂Mg_{0.5} (T3). For each treatment 20 seeds were sown 2 cm deep in the pots containing a uniform mixture of soil and farmyard manure. The optimum doses of GA₃ (10⁻⁶ M), 2.0 kg Ca and 0.5 kg Mg/ha were already determined by Khan (2008). Spray treatment of Ca and Mg was given as calcium chloride (CaCl₂) and magnesium sulphate (MgSO₄), respectively. There were four replicates for each treatment.

The following parameters were assessed in terms of height per plant, leaf area (LA) per plant, leaf area index (LAI), fresh weight (FW) per plant, dry weight (DW) per plant, net photosynthetic rate (P_N), stomatal conductance (gs), carboxylation efficiency (CE), water use efficiency (WUE), leaf carbonic anhydrase (CA) activity, leaf chlorophyll (Chl) content and leaf nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) contents at 60 and 75 DAS and capsules per plant, seeds per capsule, 1,000-seed weight, seed yield per plant, biological yield per plant, harvest

index, oil content, oil yield per plant, iodine value, fibre yield per plant and lodging at harvest.

Determination of growth characteristics

LA per plant was measured with the help of a graph paper. The area of three leaves (upper, middle and lower) of each plant from each treatment was determined and LAI, using the formula proposed by Watson (1958). DW was recorded after drying the plants at 80°C for 24 h in a hot air oven.

Determination of physiological and biochemical parameters

P_N and gs were measured in cloudless clear days at light-saturating intensity between 11.00 and 12.00 h on the uppermost, fully expanded leaves, using Infrared Gas Analyser (IRGA), portable photosynthesis system (LI-COR Lincoln, NE, USA). CE and WUE were determined by adopting the following formula (Tiwari et al. 1998):

$$CE = \frac{P_N (\mu\text{mol}(\text{CO}_2)\text{m}^{-2}\text{s}^{-1})}{\text{Internal CO}_2 (\text{ppm})}$$

$$WUE = \frac{P_N (\mu\text{mol}(\text{CO}_2)\text{m}^{-2}\text{s}^{-1})}{\text{Transpiration rate (mmole H}_2\text{O m}^{-2}\text{s}^{-1})}$$

The activity of CA (E.C.4.2.1.1) enzyme was determined as given by Dwivedi and Randhawa (1974). The leaf samples were cut into small pieces and suspended in cysteine hydrochloride solution. The samples were incubated at 4°C for 20 min. The pieces were blotted and transferred to the test tubes containing phosphate buffer (pH 6.8), followed by the addition of alkaline bicarbonate solution and bromothymol blue indicator. The test tubes were incubated at 5°C for 20 min. After addition of 0.2 ml of methyl red indicator, the reaction mixture was titrated against 0.05 N HCl. The results are expressed as $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ leaf fresh mass s}^{-1}$.

Fresh leaf samples, taken from the youngest fully expanded leaves, were extracted with 80% acetone and the absorbance was recorded at 663 and 645 nm using the spectrophotometer (Spectronic 20D, Milton Roy, USA). Leaf Chl content was determined in terms of mg g⁻¹ FW by using the formula of Arnon (1949).

For leaf -N, -P, -K, -Ca and -Mg concentration, dried plant material was milled to pass through a 0.42 mm sieve, and 100 mg samples were analysed by a Kjeldahl method, which included 0.5% selenium as a catalyst and salicylic acid to reduce nitrate (Eastin 1978). The total leaf -N and -P concentration was determined according to the method of Lindner (1944) and Fiske and Subba Row (1925), respectively. The method of Hesse (1971) was used to determine leaf -Ca and -Mg content. Leaf-K concentration was estimated using flame photometer (C150, AIMIL, India).

Oil was extracted by taking 25 g meal of ground seeds and transferring into a Soxhlet apparatus to which 100 mL pure petroleum ether was added. The apparatus was kept on a water bath at 60°C for 6 h. At the end of each extraction process, the petroleum extract was evaporated. The oil left after the evaporation was weighed and expressed as percentage of the mass of the seed. Oil yield was computed on the basis of seed yield and oil percentage.

Statistical analysis

Data were analyzed statistically with SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). In applying the F test, the error due to replicates was also determined. When 'F' value was found to be significant at 5% level of probability, the least significant difference (LSD) was calculated.

Results

Growth characteristics

The results exhibited that GA₃ in association with foliar spray of CaCl₂ and/or MgSO₄ significantly increased all the

growth characteristics. However, foliar spray of GA₃ with the combination of 2.0 kg Ca and 0.5 kg Mg (T3) proved more effective (Table 1). Treatments T1 and T2 increased plant height by 6.3 and 4.3% and 15.3 and 10.9%, while, T3 treatment increased plant height by 23.0 and 12.0% at 60 and 75 DAS, respectively. An increase of 37.7 and 19.4% in LA and 37.6 and 19.4% in LAI of T3 treated plants was recorded at 60 and 75 DAS, respectively than T0. At both the sampling stages maximum FW and DW were recorded in the plants sprayed with the combination of GA₃, Ca and Mg (T3). Treatment T3 enhanced FW and DW by 64.5 and 37.2% and 38.2 and 20.6% at 60 and 75 DAS, respectively. Among the genotypes tested 'Shubhra' performed well for most of the growth parameters, whereas, 'Parvati' the least (Table 1).

Physiological and biochemical parameters

The data revealed that treatment T3 improved P_N by 20.7 and 19.1% at 60 and 75 DAS, respectively (Table 2) and proved best for gs, CE, and WUE and exhibited an increase of 12.8 and 6.9%, 5.6 and 7.3% and 3.8 and 10.5% at 60 and 75 DAS, respectively. Treatment T3 also significantly ameliorated the activity of CA enzyme which is reflected

Table 1 Effect of GA₃, Ca and Mg on plant height, leaf area, leaf area index and fresh and dry weights of linseed genotypes at 60 and 75 DAS. LSD ($p \leq 0.05$) was employed to separate the means in the table

Treatments (T)	Cultivars (Cv)	Plant height (cm)		Leaf area per plant (cm ²)		Leaf area index		Fresh weight per plant (g)		Dry weight per plant (g)	
		60	75	60	75	60	75	60	75	60	75
T0 (control)	Parvati	51.6	59.3	163.5	427.3	3.33	8.70	15.3	25.1	3.62	6.29
	Shekhar	54.4	69.5	170.3	456.4	3.47	9.29	14.5	24.4	4.13	6.58
	Shubhra	59.5	65.7	194.4	470.5	3.96	9.58	18.6	27.0	4.96	7.62
	Mean	55.2	64.8	176.1	451.4	3.59	9.19	16.1	25.5	4.24	6.83
T1	Parvati	63.7	68.0	220.2	517.7	4.48	10.54	22.1	32.7	4.90	7.06
	Shekhar	59.2	74.2	237.3	539.6	4.83	10.99	20.5	30.2	5.71	7.49
	Shubhra	68.3	73.5	248.0	569.4	5.05	11.59	27.3	37.7	6.19	8.74
	Mean	55.2	71.9	235.2	542.2	4.79	11.04	23.3	33.5	5.60	7.76
T2	Parvati	53.5	62.5	188.4	469.4	3.84	9.56	19.4	29.4	4.18	6.71
	Shekhar	58.9	71.7	196.3	490.2	4.00	9.98	17.8	28.5	4.60	7.32
	Shubhra	63.6	68.6	217.5	518.0	4.43	10.55	22.2	33.3	5.44	7.96
	Mean	58.7	67.6	200.7	492.5	4.09	10.03	19.8	30.4	4.74	7.33
T3	Parvati	63.4	65.6	231.0	508.3	4.70	10.35	24.6	32.5	5.23	7.69
	Shekhar	67.4	77.4	238.5	541.5	4.86	11.03	23.8	32.5	5.84	8.21
	Shubhra	72.8	74.8	257.6	566.5	5.25	11.54	31.2	39.9	6.50	8.81
	Mean	67.9	72.6	242.4	538.8	4.94	10.97	26.5	35.0	5.86	8.24
LSD at 5%	T	3.00	2.34	5.50	4.58	0.20	0.16	2.28	1.83	0.13	0.20
	Cv	2.60	2.03	4.77	4.20	0.18	0.14	1.98	1.59	0.12	0.17
	T × Cv	5.19	4.06	9.53	8.40	0.35	0.27	3.95	3.17	0.23	0.34

Each value is mean of four replicates. [(i) 10^{-6} M GA₃ + 0 kg Ca + 0 kg Mg/ha (T0, Control), (ii) 10^{-6} M GA₃ + Ca₂Mg₀ (T1), (iii) 10^{-6} M GA₃ + Ca₀Mg_{0.5} (T2) and (iv) 10^{-6} M GA₃ + Ca₂Mg_{0.5} (T3)]

Table 2 Effect of GA₃, Ca and Mg on net photosynthetic rate (P_N), stomatal conductance (g_s), carboxylation efficiency (CE), water use efficiency (WUE) and carbonic anhydrase (CA) activity of linseed genotypes at 60 and 75 DAS. LSD ($p \leq 0.05$) was employed to separate the means in the table

Treatments (T)	Cultivars (Cv)	P_N [$\mu\text{mol (CO}_2\text{) m}^{-2} \text{ s}^{-1}$]		g_s [$\mu\text{mol (H}_2\text{O) m}^{-2} \text{ s}^{-1}$]		CE		WUE		CA [$\mu\text{mol (CO}_2\text{) kg}^{-1}$ (f.m.) s^{-1}]	
		60	75	60	75	60	75	60	75	60	75
T0 (control)	Parvati	18.5	20.2	3.10	3.60	0.036	0.041	12.51	14.16	372.6	452.3
	Shekhar	18.7	20.8	3.20	3.50	0.033	0.040	12.58	15.62	381.3	463.9
	Shubhra	18.6	21.6	3.10	4.20	0.038	0.043	12.62	15.87	397.5	472.2
	Mean	18.6	20.9	3.13	3.77	0.036	0.041	12.57	15.22	383.8	462.8
T1	Parvati	19.3	21.8	3.30	3.50	0.037	0.042	12.68	15.39	409.2	491.7
	Shekhar	19.7	22.4	3.30	3.60	0.035	0.043	12.66	15.56	421.4	507.9
	Shubhra	20.2	23.1	3.40	4.40	0.039	0.044	12.71	16.90	446.7	519.8
	Mean	19.7	22.4	3.33	3.83	0.037	0.043	12.68	15.61	425.8	506.5
T2	Parvati	22.2	24.3	3.60	3.90	0.039	0.044	13.17	15.97	431.0	503.5
	Shekhar	22.1	25.2	3.70	3.80	0.038	0.045	12.96	16.10	449.2	521.3
	Shubhra	23.5	25.7	3.80	4.60	0.042	0.047	13.67	18.86	471.2	541.3
	Mean	22.6	25.1	3.70	4.10	0.040	0.045	13.27	16.98	450.5	522.0
T3	Parvati	22.1	24.1	3.40	3.80	0.037	0.043	12.88	15.86	451.3	517.2
	Shekhar	21.8	25.2	3.50	3.70	0.036	0.045	12.75	16.06	463.5	531.1
	Shubhra	23.5	25.3	3.70	4.60	0.040	0.046	13.51	18.50	488.1	553.2
	Mean	22.5	24.9	3.53	4.03	0.038	0.044	13.05	16.81	467.6	533.8
LSD at 5%	T	0.72	0.69	0.17	0.21	0.001	0.002	0.44	0.47	11.24	7.15
	Cv	0.63	0.60	0.15	0.19	0.001	0.002	0.38	0.41	9.74	6.20
	T $\times$ Cv	1.25	1.19	0.29	0.37	0.003	0.004	0.76	0.81	19.47	12.39

Each value is mean of four replicates. [(i) 10^{-6} M GA₃ + 0 kg Ca + 0 kg Mg/ha (T0, Control), (ii) 10^{-6} M GA₃ + Ca₂Mg_{0.5} (T1), (iii) 10^{-6} M GA₃ + Ca₀Mg_{0.5} (T2) and (iv) 10^{-6} M GA₃ + Ca₂Mg_{0.5} (T3)]

by an increase of 21.8 and 15.3% at 60 and 75 DAS, respectively over the plants treated with GA₃ alone (Table 2). Highest value for leaf Chl content was recorded in the plants sprayed with T2 (GA₃ + Ca₀Mg_{0.5}), which enhanced Chl content by 30.3 and 25.9% at 60 and 75 DAS, respectively than T0 (Table 3). The effect of treatments on leaf-N content was not significant at each sampling stage (Table 3). However, a significant effect of treatments was noted on leaf -P and -K content at 60 DAS only and -Ca and -Mg content at both the stages (Table 3). It is noteworthy that spray treatments T1 and T3 increased the concentration of leaf-P, -K and -Ca content at each sampling stage. On the other hand, a decrease from control was recorded in the levels of these nutrients in T2 (GA₃ + Ca₀Mg_{0.5}) treated plants (Table 3). At both the sampling stages, highest concentration of Ca and Mg was recorded in the plants sprayed with T1 and T2, respectively. T1 treatment enhanced Ca content by 48.7 and 40.4%. T2 improved Mg content by 30.8 and 28.6% at 60 and 75 DAS, respectively.

Regarding genotype differences, ‘Shubhra’ performed well and gave maximum values for most of the parameters.

‘Shubhra’ showed 4.7 and 5.9%, 8.4 and 6.2%, and 3.5 and 3.3% higher values for P_N , CA activity and leaf Chl content at 60 and 75 DAS, respectively than ‘Parvati’ (Tables 2 and 3). On the other hand, genotypes did not differ with respect to leaf-N content at both stages and leaf -P and -K content at 75 DAS. Among the interactions, T3 $\times$ ‘Shubhra’ interacted best and gave maximum values for most of the physiological and biochemical parameters. This interaction improved P_N by 27.1 and 25.1%, g_s by 19.4 and 9.5%, CE by 5.3 and 7.0%, WUE by 7.1 and 16.5%, CA by 31.0 and 22.3% and Chl by 33.2 and 28.8% at 60 and 75 DAS, respectively than T0 $\times$ ‘Parvati’ (Tables 2 and 3).

Yield and quality characteristics

The results showed that the effect of treatments and their interactions was significant on capsules per plant, 1,000-seed weight, seed yield, biological yield, harvest index, oil yield, fibre yield and lodging. However, the effect of treatments and their interactions on seeds per capsule, oil content and iodine value was not significant. Application of GA₃ in combination with Ca₂Mg_{0.5} (T3) proved best and

Table 3 Effect of GA₃, Ca and Mg on chlorophyll and leaf -N, -P, -K, -Ca and -Mg content of linseed genotypes at 60 and 75 DAS. LSD ($p \leq 0.05$) was employed to separate the means in the table

Treatments (T)	Cultivars (Cv)	Chlorophyll content (mg g ⁻¹ leaf f.m.)		Leaf-N content (%)		Leaf-P content (%)		Leaf-K content (%)		Leaf-Ca content (%)		Leaf-Mg content (%)	
		60	75	60	75	60	75	60	75	60	75	60	75
T0 (control)	Parvati	1.32	1.46	2.44	2.86	0.26	0.27	2.49	2.62	0.36	0.43	0.23	0.31
	Shekhar	1.31	1.46	2.53	2.88	0.26	0.27	2.42	2.60	0.39	0.46	0.26	0.36
	Shubhra	1.34	1.48	2.60	3.06	0.27	0.28	2.57	2.72	0.42	0.51	0.29	0.38
	Mean	1.32	1.47	2.52	2.93	0.26	0.27	2.49	2.65	0.39	0.47	0.26	0.35
T1	Parvati	1.45	1.64	2.61	2.94	0.27	0.28	2.59	2.68	0.52	0.60	0.20	0.27
	Shekhar	1.46	1.63	2.75	2.93	0.26	0.28	2.51	2.71	0.58	0.65	0.24	0.32
	Shubhra	1.49	1.66	2.86	3.17	0.28	0.29	2.69	2.80	0.63	0.73	0.26	0.35
	Mean	1.47	1.65	2.74	3.01	0.27	0.28	2.60	2.73	0.58	0.66	0.23	0.31
T2	Parvati	1.68	1.80	2.31	2.72	0.25	0.27	2.41	2.52	0.34	0.40	0.30	0.43
	Shekhar	1.70	1.86	2.42	2.76	0.25	0.27	2.34	2.48	0.37	0.42	0.34	0.45
	Shubhra	1.76	1.89	2.51	2.97	0.26	0.28	2.49	2.61	0.39	0.47	0.37	0.48
	Mean	1.72	1.85	2.41	2.82	0.25	0.27	2.41	2.54	0.37	0.43	0.34	0.45
T3	Parvati	1.68	1.79	2.76	3.24	0.27	0.28	2.63	2.81	0.42	0.52	0.27	0.35
	Shekhar	1.69	1.86	2.98	3.20	0.27	0.29	2.60	2.74	0.49	0.57	0.32	0.39
	Shubhra	1.75	1.88	3.14	3.32	0.29	0.31	2.72	2.86	0.54	0.61	0.34	0.41
	Mean	1.71	1.84	2.96	3.25	0.28	0.29	2.65	2.80	0.48	0.57	0.31	0.38
LSD at 5%	T	0.04	0.02	NS	NS	0.01	NS	0.07	NS	0.05	0.04	0.05	0.05
	Cv	0.03	0.02	NS	NS	0.01	NS	0.06	NS	0.05	0.04	0.04	0.04
	T × Cv	0.07	0.04	NS	NS	0.02	NS	0.12	NS	0.09	0.07	0.07	0.08

Each value is mean of four replicates. [(i) 10^{-6} M GA₃ + 0 kg Ca + 0 kg Mg/ha (T0, Control), (ii) 10^{-6} M GA₃ + Ca₂Mg₀ (T1), (iii) 10^{-6} M GA₃ + Ca₀Mg_{0.5} (T2) and (iv) 10^{-6} M GA₃ + Ca₂Mg_{0.5} (T3)]; NS = non-significant

produced 76.0% more capsules exceeding the control. T3 improved 1,000-seed weight, seed yield, biological yield, harvest index, and oil yield by 5.4, 39.6, 33.5, 4.4, and 46.9%, respectively (Tables 4 and 5). Regarding fibre yield, control plants produced lowest fibre and T3 the highest. Treatment T3 increased fibre yield by 36.9% while this increase was only 28.0 and 25.0% in T2 and T1 treated plants, respectively (Table 5). Maximum lodging was recorded in control plants, however, the value given by control was at par with that of T2 treatment. Combined application of GA₃, CaCl₂ and MgSO₄ (T3) proved most effective in reducing the lodging than any other combination (Table 5).

Tested genotypes showed a significant difference in yield and quality parameters, except biological yield and iodine value. 'Shubhra' gave 16.1, 1.3, 7.4, 14.1, 6.2, 4.6, 19.8 and 5.8% more capsules, seeds per capsule, 1,000-seed weight, seed yield, harvest index, oil content, oil yield and fibre yield, respectively than 'Parvati' (Tables 4 and 5). Plants of cultivar 'Shubhra' exhibited a reduction in lodging by 24.7% than 'Parvati' which lodged maximum. Of the genotypes tested 'Shubhra' performed well and 'Parvati' the least (Table 5). Interaction T3 × 'Shubhra'

proved best for most of the yield and quality attributes with the exception of seeds per capsule, oil content and iodine value (Tables 4 and 5).

Discussion

The results showed that application of GA₃ with CaCl₂ or MgSO₄ alone as well as in combination improved most of the growth, physiological, biochemical, yield and quality attributes.

Growth characteristics

The foliar application of GA₃ with CaCl₂ and/or MgSO₄ ameliorated plant height, LA, LAI and FW and DW of plants at 60 and 75 DAS. However, combined application of GA₃ with CaCl₂ and MgSO₄ (T3) was more effective. The increase in plant growth in response to GA₃, CaCl₂ and MgSO₄ may be explained on the basis of their role in seed germination, cell division and cell elongation (Hirschi 2004) resulting in better growth and development of plant body (Cakmak and Kirkby 2008). Ca and Mg are

Table 4 Effect of GA₃, Ca and Mg on capsules per plant, seeds per capsule, 1,000-seed weight and seed and biological yield of linseed genotypes at harvest. LSD ($p \leq 0.05$) was employed to separate the means in the table

Treatments (T)	Cultivars (Cv)	Capsules per plant	Seeds per capsule	1,000-seed weight (g)	Seed yield per plant (g)	Biological yield per plant (g)
T0 (control)	Parvati	73.0	8.60	8.60	2.71	10.61
	Shekhar	85.0	7.72	9.07	2.93	11.45
	Shubhra	80.7	8.57	9.36	3.15	12.02
	Mean	79.6	8.30	9.01	2.93	11.36
T1	Parvati	119.3	8.66	9.11	3.40	13.21
	Shekhar	126.7	7.93	9.44	3.51	13.34
	Shubhra	139.0	8.83	9.71	3.78	13.69
	Mean	128.3	8.47	9.42	3.56	13.41
T2	Parvati	112.7	8.64	8.86	3.23	12.52
	Shekhar	119.3	8.11	9.35	3.37	12.85
	Shubhra	131.7	8.50	9.55	3.61	13.11
	Mean	121.2	8.42	9.25	3.40	12.83
T3	Parvati	129.0	8.75	9.23	3.74	14.33
	Shekhar	138.7	8.26	9.46	4.15	15.71
	Shubhra	152.3	9.17	9.82	4.37	15.47
	Mean	140.0	8.73	9.50	4.09	15.17
LSD at 5%	T	5.65	NS	0.22	0.17	1.23
	Cv	4.90	0.74	0.19	0.15	NS
	T × Cv	9.79	NS	0.38	0.29	2.13

Each value is mean of four replicates. [(i) 10^{-6} M GA₃ + 0 kg Ca + 0 kg Mg/ha (T0, Control), (ii) 10^{-6} M GA₃ + Ca₂Mg₀ (T1), (iii) 10^{-6} M GA₃ + Ca₀Mg_{0.5} (T2) and (iv) 10^{-6} M GA₃ + Ca₂Mg_{0.5} (T3)]; NS = non-significant

components of many metabolically active compounds and play important role in physiological and biochemical functions. Ca acts as an activator of many enzymes, help in figuration of growing tips of roots and shoots, involved in the availability of other nutrients, mitotic cell division, cell elongation, tissue extensibility, stimulates utilization of carbohydrates and the process of photosynthesis (Gardner et al. 2003; Hirschi 2004; Mukherji and Ghosh 2005). Gibberellins promote growth through cell expansion by stimulating the destruction of growth-repressing proteins (Achard et al. 2009). Similarly, Mg being the constituent of the chlorophyll molecule, acts as a bridging element for the aggregation of ribosomes, plays a key role in photosynthesis and is essential for the activity of many enzymes involved in photosynthesis (Mengel and Kirkby 1996; Cakmak and Kirkby 2008). Thus, Ca and Mg take part directly or indirectly in the process of development of shoot and leaf, hence enhancement in plant height and leaf area and better orientation of leaves for harvesting maximum solar energy (Table 1).

Physiological and biochemical parameters

The results exhibited that application of GA₃ in association with CaCl₂ and MgSO₄ was more effective that significantly

enhanced P_N and CA activity in all the linseed genotypes. The enhanced activity of CA might have led to increased P_N conceivably through a rapid reversible hydration of CO₂ maintaining its constant supply to ribulose biphosphate carboxylase in the stroma of chloroplast (Badger and Price 1994). The ready availability of the sprayed nutrients at the site of their metabolism, and the dependence of the activity of several enzymes and regulatory processes on these nutrients (Mengel and Kirkby 1996; Hirschi 2004; Mukherji and Ghosh 2005) ultimately resulted in higher level of activity. Khan et al. (2010) in linseed and Naeem et al. (2009) in *Cassia sophera* L. reported increased activity of CA by the application of GA₃ and CaCl₂.

Magnesium plays a major role in carboxylation process of RuBP-driving CO₂ fixation (Portis 1992) and activation of enzyme phosphoenol pyruvate (PEP) carboxylase accelerated incorporation of CO₂ into PEP (Wedding and Black 1988), photosynthetic electron transport in PS I and PS II reaction centres that absorb photons and initiate electron flow (Cakmak and Kirkby 2008). An increase in Mg supply is associated with enhanced ATP formation (Shabala and Hariadi 2005), thereby making Mg treated plants with high energy status. The cumulative response of all these set the plants to perform more efficient photosynthesis, which is witnessed by enhanced dry matter. The data showed increased values of gs in GA₃,

Table 5 Effect of GA₃, Ca and Mg on harvest index, oil content, oil yield, iodine value, fibre yield and lodging of linseed genotypes at harvest. LSD ($p \leq 0.05$) was employed to separate the means in the table

Treatments (T)	Cultivars (Cv)	Harvest index (%)	Oil content (%)	Oil yield per plant (g)	Iodine value	Fibre yield per plant (g)	Lodging (%)
T0 (control)	Parvati	25.5	38.2	1.03	181.6	1.31	33.7
	Shekhar	25.6	38.1	1.12	187.4	1.37	30.0
	Shubhra	26.2	39.5	1.24	183.4	1.39	29.0
	Mean	25.8	38.6	1.13	184.1	1.36	30.9
T1	Parvati	25.7	38.3	1.30	186.6	1.62	32.0
	Shekhar	26.3	39.5	1.39	184.3	1.66	24.7
	Shubhra	27.6	39.7	1.50	186.6	1.82	26.3
	Mean	26.6	39.2	1.40	185.8	1.70	27.7
T2	Parvati	25.8	39.1	1.26	189.0	1.72	34.3
	Shekhar	26.2	40.3	1.36	194.5	1.74	29.3
	Shubhra	27.5	41.3	1.49	182.6	1.75	27.7
	Mean	26.5	40.2	1.37	188.7	1.74	30.4
T3	Parvati	26.1	39.2	1.46	194.3	1.82	31.0
	Shekhar	26.4	39.9	1.72	206.0	1.86	26.7
	Shubhra	28.2	41.3	1.81	189.3	1.89	22.0
	Mean	26.9	40.1	1.66	196.5	1.86	26.6
LSD at 5%	T	0.69	NS	0.14	NS	0.05	1.90
	Cv	0.60	1.66	0.13	NS	0.04	1.82
	T × Cv	1.19	NS	0.25	NS	0.08	3.63

Each value is mean of four replicates. [(i) 10^{-6} M GA₃ + 0 kg Ca + 0 kg Mg/ha (T0, Control), (ii) 10^{-6} M GA₃ + Ca₂Mg₀ (T1), (iii) 10^{-6} M GA₃ + Ca₀Mg_{0.5} (T2) and (iv) 10^{-6} M GA₃ + Ca₂Mg_{0.5} (T3)]; NS = non-significant

CaCl₂ and/or MgSO₄ treated plants. The higher values of gs in treated plants can be explained on the basis of the role of absorbed nutrients particularly K. The absorbed potassium plays a role in stomatal movement and influences water balance of plants (Hugouvieux et al. 2002). However, the data (Table 2) revealed that gs is unable to keep pace with the increasing levels of P_N in treated plants which is reflected by higher values for WUE. Application of GA₃ in combination with CaCl₂ and MgSO₄ showed a significant increase in Chl content. Afroz et al. (2005) also reported an improvement in Chl content in GA₃ treated mustard plants. Increased values for leaf Chl content may be due to suppressed retardation of chlorophyll by the application of Ca (Hepler 2005) and enhanced biosynthesis by supplementary Mg which serves as the structural component of chlorophyll molecule and needed for its biosynthesis. Magnesium chelatase is an important enzyme catalysing the first step of chlorophyll biosynthesis by inserting Mg⁺² into protoporphyrin IX (Walker and Weinstein 1991). It appears that foliar spray of hormone along with the nutrients at the crucial stage enhanced photosynthetic efficiency.

The data of leaf analyses showed a decrease in leaf -K and -Ca content due to effect of T2 and in leaf Mg content due to T1 treatment (Table 3) which can be attributed to their mutual antagonistic effect. These results agree with the

findings of Stein (1990) and Hille (1992). However, a higher concentration of nutrients was recorded in the plants supplemented with the combination of GA₃, CaCl₂ and MgSO₄ as compared to their individual application. In fact, calcium is reported to enhance availability and uptake of nutrients and also increased nitrogen use efficiency (Easterwood 2002). As nutrients are components of many metabolically active compounds and participate in several physiological and biochemical functions (Marschner 2002), their enhanced content in plants may have directly or indirectly helped in enhancing the crop performance.

Yield and quality attributes

Foliar application of GA₃ alone as well as in combination with CaCl₂ or MgSO₄ accelerated most of the yield and quality attributes of linseed genotypes. However, their combined application proved more effective in ameliorating yield and quality parameters (Tables 4 and 5). As shown in Table 1, T3 treatment resulted in taller plants with increased vegetative growth; plants with increased height bear more number of branches which in turn produce more capsules. Furthermore, enhanced vegetative growth resulted in higher demand for absorption of nutrients and water which might have enhanced the yield of the crop in terms of increased

number of capsules and subsequently seed and oil yield. The increased seed yield of treated plants seems to be mainly responsible for the observed higher oil yield. Increase in capsule number may be traced to various roles of GA₃, particularly in differentiation (Huttly and Phillips 1995; Afroz et al. 2005) leading to enhanced number of flowers and fruit set; cell division and cell enlargement (Arteca 1996; Marschner 2002). As mentioned earlier, application of CaCl₂ and MgSO₄ along with GA₃ increased gs, CE, WUE, CA activity and chl content. Higher values of these parameters might have cumulative positive effect on P_N providing sufficient photosynthates. Enormous availability of photosynthates favourably affected their partitioning to develop sink which is confirmed by higher values for capsule number and harvest index. Moreover, transport and utilization of photosynthates is greatly influenced by Mg, thereby markedly influencing carbohydrate partitioning between source and sink (Cakmak and Kirkby 2008) which ultimately contributed to higher crop yield.

The observed increase in fibre yield may be explained on the basis of the role of GA₃ in internodal elongation that resulted in the development of plants with longer stem which gave more and fine fibres. Our earlier results (Khan et al. 2009) showed that increased plant height of GA₃ treated plants causes lodging of plants which severely affected seed and fibre yield. However, in the present study inclusion of CaCl₂ and MgSO₄ proved effective for making the fibres stronger which reduced lodging that may also be one of the reasons for improved yield and productivity. Besides GA₃, Ca and Mg also proved helpful in providing mechanical support to the plants by strengthening the middle lamellae of stem cells in the form of their pectates. Calcium by binding to phospholipids stabilizes lipid bilayers thereby providing structural integrity to cellular membranes. This proposition is further confirmed by reduced lodging and increased fibre production in the treated plants (Table 5).

Conclusion

On the basis of assessment of results, it can be concluded that application of GA₃ with CaCl₂ and MgSO₄ is more efficient than their application alone. Combined application improved photosynthetic parameters including Chl content with a parallel increase in CA activity which also enhanced nutrient use-efficiency. The cumulative effect ultimately contributed to enhanced dry matter accumulation. Thus, it can be postulated that combined application of GA₃, CaCl₂ and MgSO₄ is more effective to ameliorate seed yield without compromising fibre production coupled with reduced lodging and increased dry matter accumulation. Among the genotypes tested 'Shubhra' performed well and

gave highest seed and fibre yields. Therefore, combined application of GA₃, Ca and Mg proved helpful in exploring maximum potential of linseed genotypes, hence this combination can be used as a remedy for cost effective cultivation of linseed crop with good yield of both seed and fibre.

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