Response of canola (*Brassica napus* L.) to exogenous application of nitrogen, salicylic acid and gibberellic acid under an arid climate

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Abstract

*Sustainable improvement of yield under limited land resources is a challenge for future food security and types and techniques of fertilization are critical for this challenge. In this view, a 2-year study was conducted to investigate the interaction of nitrogen (N) with salicylic acid and gibberellic acid on productivity and quality of canola (*Brassica napus* L.) during the year 2014-15 and 2015-16. The experiment was conducted in a split-plot arrangement of treatments where four N levels (60, 80, 100, 120 kg ha⁻¹), kept in main plots, and four levels of plant growth regulators PGRs [Control, salicylic acid (100 ppm), gibberellic acid (100 ppm) and combination of the two], kept in sub-plots, and replicated three times. The results indicated that combined application of N and PGRs in either combination improved the morphological/yield parameters, seed yield and seed quality attributes of canola crop. The maximum seed yield of 2230 kg ha⁻¹ was recorded when N application @ 120 kg ha⁻¹ interacted with combine application of salicylic acid and gibberellic acid. The combined application of gibberellic acid and salicylic acid enhanced the seed yield and seed oil contents of canola by 13.3-17.7% and 28.9-29.8%, respectively, than control treatment (no application of salicylic acid and gibberellic acid) during the both years of experimentation. Therefore, to combat emerging food security issues, combined application of salicylic acid and gibberellic acid along with N (at 100/120 kg ha⁻¹) will be a practicable option to improve the productivity and quality of canola grown under arid climates.*

Keywords: Canola nutrition, nitrogen, gibberellic acid, salicylic acid, oil contents, protein contents

Introduction

With limited resources of land and water, it is critical to produce sustainable agriculture. Regarding oil seed crops, Pakistan is ranked as the fourth largest importer of the edible oil in the world whose almost 75% edible oil demand is fulfilled through imports (Zaidi, 2014). Pakistan imports ~1.8 million tons of the edible oil of worth almost US\$ 1.4 billion each year (Government of Pakistan, 2015-16). This indicates that there is huge shortage of edible oil in the country which can only be fulfilled by using new techniques in agriculture.

Although, the crops such as mustard, sunflower and cotton contribute in the domestic production of edible oil; nonetheless their share is not enough to fulfill the edible oil needs of the population of the country (Zaidi, 2014). There are various factors which are responsible for low edible oil production in the country. Firstly, the oilseed crops are grown on a very small area and thus the overall oilseed production is not enough to extract the required quantity of

the edible oil. Secondly, the oilseeds crops are grown on marginal lands due to which per acre yield of the oilseed crops is very low which further deteriorate this situation (Ali *et al.*, 2014). Indeed, the planation period of the main oilseed crops (e.g. rapeseed and mustard) is the same as of other rabi crops (e.g. wheat, gram) and thus the farmers never compromise to sacrifice their main crops for oilseed crops. Thirdly, the insect pests (e.g. aphid) and diseases are also a hindrance in harvesting the optimum yield of oilseeds. Unwise use of fertilizers is also a major reason for low oilseed production in the country.

Among the oilseed crops, canola (*Brassica napus* L.) has emerged as an important oilseed crop in the world including Pakistan. However, the yield of canola is low in Pakistan due to poor nutrient management. Thus, to optimize the production of canola in the country, wise nutrient management strategies might be quite useful to boost up its production under arid and semi-arid environment.

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The oilseed crops require adequate nitrogen (N) supply for attaining the maximum productivity (Stahl *et al.*, 2017; Gu *et al.*, 2018). Thus, the improvement in N use efficiency of oilseed crop required the optimization of the rates of N fertilizer. Various factors such as the soil N supply and moisture fluctuations at the critical growth stages of oilseed crops may impact the N use efficiency.

Among the rapeseed species, the N requirement of canola is higher than others and it is considered as a high N demanding crop (Rathke *et al.*, 2005; Stahl *et al.*, 2017). Thus, it is critical to optimize the N application to exploit the full potential of canola crop. Indeed, N is an essential component of amino acids, the building blocks of proteins. N is also an integral part of the green pigments (e.g. chlorophyll) of all the plants and triggers the process of photosynthesis. The other roles of N in plants include its involvement in seed germination, leaf development and

Materials and Methods

Experimental site and soil

Two year experiment was carried out at the Agronomic Research Area, The Islamia University of Bahawalpur (longitude 71.4° E, latitude 29° N, and altitude 181 masl) during 2014-15 and 2015-16. Prior to sowing of canola crop, the soil samples (from 0-20 cm depth) were collected with the help of an auger. These soil samples were placed into tagged polythene bags and were sent to soil and water testing laboratory of the Regional Agriculture Research Institute (RARI) Bahawalpur for analysis. The soil analysis report of the experimental soil showed that the soil was loamy in texture with an average pH of 8.2-8.4, electrical conductivity of 247-250 $\mu\text{S cm}^{-1}$, exchangeable sodium of 0.23-0.24%, available phosphorus of 7-9 ppm, available potassium of 115-121 ppm, total N of 0.05-0.08 % and

Table 1: Rainfall and temperature data during the study period

	Temperature ($^{\circ}\text{C}$)				Rainfall (mm)	
	Maximum		Minimum		2014-15	2015-16
	2014-15	2015-16	2014-15	2015-16		
November	32.4	31.8	9.7	9.9	0.00	0.00
December	25.5	28.0	1.7	2.5	0.00	0.00
January	24.3	26.0	2.2	1.9	0.00	0.00
February	27.5	33.1	6.9	5.5	16.5	4.20
March	38.2	37.5	8.1	12.3	24.4	29.7

flower/fruit development (Kumar *et al.*, 2018; Saini *et al.*, 2017).

Likewise, the use of plant growth regulators (PGRs) viz. cytokinin, salicylic acid, and abscisic acid is very useful for improving the crop growth under optimal and suboptimal conditions (Farooq *et al.*, 2009; Upreti and Maryada, 2016). Among these, the role of salicylic acid in multiple physiological processes of plants including growth, nutrient uptake, flower induction, stomatal movements, enzyme activities and photosynthesis is well-known (Hayat *et al.*, 2007). On the other hand, gibberellic acid is also an important growth regulator which affects the growth and yield (Rafeekher *et al.*, 2002), through positive influence on stem and internode elongation, seed germination, enzyme activation and fruit setting (Hussain *et al.*, 2018).

As the oilseeds are mostly grown under arid and semi-arid climates in Pakistan without any supplemental irrigation, it was hypothesized that the salicylic and gibberellic acid may interact with N to improve the seed yield and quality of canola. Thus, major objective of the study was to check the interactive effect of N with salicylic acid and gibberellic acid on growth, seed yield and seed quality of canola.

saturation percentage of 35-37% during both years. Data on temperature and rainfall during both experimental sites during the growth period is given in Table 1.

Experimental design and treatments

The experiment was laid out in randomized complete block design, in split plot arrangement with a net plot size of 2 m $\times$ 3 m. The experiment comprised of four N levels [120 (high), 100 (recommended), 80 (medium) and 60 (low) kg ha^{-1}], kept in main plots, and four PGRs treatments viz., salicylic acid (100 ppm), gibberellic acid (100 ppm), salicylic acid + gibberellic acid; no PGRs application being taken as control. The foliar application of PGRs was made twice, at 30 and 45 days after sowing. Hyola 433 was used as tested canola hybrid in this study.

Seedbed preparation, sowing and crop husbandry

Before sowing of canola crop in each year, field was irrigated to bring the soil at field capacity. When the soil reached at workable soil moisture, the seed bed was prepared through 3 cultivations (using tractor mounted cultivator up to depth of 0.20 m), which was followed by a

single planking with wooden planker. The canola crop was sown on November 02 and November 05 during 2014 and 2015, respectively. The canola crop was seeded at 5 kg ha⁻¹ in 75 cm apart rows with the help of a locally manufactured manual hand drill. After uniform emergence, the thinning was carried out to maintain the plant to plant distance of 15 cm. On the basis of soil analysis report, synthetic fertilizers were applied at the rate of 60:50 kg P₂O₅ and K₂O, respectively using the diammonium phosphate (46% P, 18% N) and sulphate of potash (50% K₂O) as sources of P and K, respectively. Full dose of P and K was applied at sowing time. Half of the N [using urea (46% N) as source] from each treatment was used as basal dose; the remaining N in each treatment was splitted into two halves (applied with first and second irrigation). The N available from diammonium phosphate was added to each treatment when applying the urea in each experimental N treatment. In total, four irrigations (each of 3 acre inches) were applied to canola crop during each year. The first irrigation was done 21 days after emergence. The second, third and fourth irrigations were applied at vegetative, head formation, and seed filling stages, respectively. The irrigation was stopped one week prior to harvesting at physiological maturity. The weeds within the experimental plots were removed through manual weeding. All the other agronomic practices and plant protection measures were adopted uniformly in all the plots to keep the crop healthy and free of insects and diseases.

Harvesting and data recording

Morphological and yield parameters

Data on morphological (plant height, leaf area index, primary/secondary branches per plant), yield parameters (plant population, 1000-seed weight, seeds per silique, silique per plant, seed yield, seed oil and protein contents) were recorded following the standard procedures. The final plant population was counted from a unit area (measuring 1 m × 1 m) at harvest from two locations of each experimental plot and was averaged. The plant height of ten plants (randomly selected) from each of the experimental plot was recorded from soil surface to the tip of plant with the help of a meter rod, and then averaged. The leaf area was measured at fifteen days by using a leaf area meter. The sampling for leaf area measurement was started twenty days after sowing (DAS) until crop harvest. After that, the leaf area index was calculated by using the formula given by Watson (1947). The number of primary and secondary branches of ten randomly selected plants from each of the experimental plots were counted, averaged and were reported separately as primary and secondary branches. At harvest maturity, the number of siliques from ten randomly selected plants were counted and then averaged to work out number of siliques

per plant. Twenty five siliques were randomly taken from five randomly selected plants from each plot and their seeds were counted.

The canola crop was harvested at harvest maturity (on April 18, 2015 and April 20, 2016) when the pods were turned from green to yellowish brown. The whole plants within an experimental plot were harvested using a locally manufactured hand sickle and the samples were left in the field for a week for sun-drying. After sun-drying, the crop samples were threshed manually to separate the seeds from stover. These seeds were weighed on an electric balance separately for each experimental plot and the seed yield was converted to kg ha⁻¹.

The 1000-seed weight was estimated after collecting three random samples of 1000 seeds from seed lot of each treatment, which were weighed on an electrical balance to find the 1000-seed weight.

Quality parameters

For protein determination, the N contents within the canola seeds were determined by Kjeldhal method (Bremner, 1964) followed by the determination of crude proteins with following formula:

$$\text{Crude protein (\%)} = \frac{(V1 - V2)N \times 14 \times 6.25 \times 100}{100 W}$$

The seed oil contents were measured by the Soxhlet Fat Extraction method (AOAC, 1990). The oil content was calculated as following:

$$\text{Oil (\%)} = \frac{\text{Weight of flask + oil} - \text{Weight of flask} \times 100}{\text{Weight of flask + seed} - \text{Weight of flask}}$$

Statistical analysis

The data collected for each parameter was manually entered in the Microsoft excel sheet followed by its analysis through the statistical software "Statistics 8.1" by using the Fischer analysis of variance technique (Steel *et al.*, 1997). The differences in the significant treatments means were then estimated by least significant difference test at 5% probability level.

Results

Morphological and yield parameters

This study indicated that N application at variable rates significantly improved the seeds per silique during both years, 1000-seed weight for first year, number of silique per plant and seed yield during the second year; results being non-significant for the remaining studied traits. Likewise, the application of PGRs significantly increased the leaf area index, primary/secondary branches, plant height, silique per

plant, seeds per silique, 1000-seed weight and seed yield during the both year of experimentation; results being non-significant for plant population during the both years. The interaction of N application with PGRs was only significant for primary branches and 1000-seed weight for first year and number of seeds per silique for second year (Tables 2, 3).

The highest siliques per plant as well as the seed yield during the second year, and the seeds per silique during the

that was statistically similar with the sole application of gibberellic acid for the secondary branches, and with the sole application of gibberellic acid and salicylic acid for 1000-seed weight during the year 2015-16 (Tables 2, 3). Combined application of gibberellic acid and salicylic acid enhanced the canola seed yield by 13.3-17.6% during both years (Table 3).

The interactive effect showed that significantly higher

Table 2: Influence of nitrogen application at variable rates in combination with plant growth regulators on allometric, morphological and yield parameters of canola

	Leaf area index		Plant population (m ²)		Plant height (cm)		Primary branches		Secondary branches		No. of silique per plant	
	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16
Nitrogen levels (N; Kg ha⁻¹)												
100	2.51	2.90	51	49	145	146	4.6	5.1	2.4	2.7	254	265AB
60	2.44	2.78	53	53	143	145	4.8	5.8	2.7	3.0	255	263B
80	2.48	2.85	55	52	144	147	4.6	5.1	2.6	2.8	257	259C
120	2.49	3.04	50	50	147	148	4.6	5.8	2.9	3.1	259	269A
LSD($p \leq 0.05$)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	2.18
Plant growth regulators (PGRs; 100 ppm)												
Control	1.8D	2.25B	48	54	139C	142B	4.1C	4.8C	1.8C	1.8C	254C	258C
SA	2.27C	2.75AB	50	48	142B	145B	4.5B	5.3B	2.6B	2.9B	257B	259B
GA	2.83B	3.10AB	51	53	145B	149A	4.8A	5.7A	2.7B	3.0AB	258B	260B
SA+GA	3.0A	3.47A	54	52	154A	150A	5.1A	5.9A	3.5A	3.7A	265A	264A
LSD($p \leq 0.05$)	0.16	0.43	NS	NS	2.69	2.14	0.52	0.38	0.32	0.77	1.49	0.58
PGR×N	NS	NS	NS	NS	NS	NS	0.76	NS	NS	NS	NS	NS

Means of nitrogen and plant growth regulators sharing the same letter, do not differ significantly at $p \leq 0.05$; SA= Salicylic acid; GA= Gibberellic acid; NS= non-significant

both years were recorded with N application at the rate of 120 kg ha⁻¹ and they were statistically similar with the N application at the rate of 100 kg ha⁻¹ (Tables 2, 3). Application of N at 80, 100 and 120 kg ha⁻¹ was equally effective for improvement in 1000-seed weight during the year 2014-15 (Table 3).

Among the PGRs, the highest leaf area index and plant height was recorded with the combined foliage application of gibberellic acid and salicylic acid during both years and that was statistically similar with sole application of gibberellic acid and salicylic acid for leaf area index and gibberellic acid for plant height during the year 2015-16 (Table 2). Sole application of gibberellic acid and combined foliage application of gibberellic acid and salicylic acid was equally effective for the improvement in the primary branches per plant during the both years of experimentation (Table 2). The highest secondary branches per plant, 1000-seed weight, seeds per silique, siliques per plant, and seed yield was recorded with the combined foliage application of gibberellic acid and salicylic acid during both years, and

number of silique per plant and seed yield for second year and number of seeds per silique during the both years were recorded with combined application of PGRs with N application @ 120 kg ha⁻¹ (Tables 2, 3). The interactive effect also showed that significantly higher 1000-seed weight for first year was recorded with combined application of PGRs with N application @ 120, 80 and 100 kg ha⁻¹, respectively (Table 3).

Quality parameters

Application of N at variable rates did not significantly affect the seed oil contents and seed protein contents. However, foliar application of PGRs significantly affected the seed oil contents and seed protein contents during both years of experimentation. The interaction of N application with PGRs was only significant for seed oil contents during the year 2014-15 (Table 3). Among the PGRs, the highest seed oil contents were recorded with the combined foliar application of gibberellic acid and salicylic acid during the year 2014-15 and 2015-16 (Table 3). Combined foliage

application of gibberellic acid and salicylic acid enhanced the seed oil contents by 28.9-29.8% than control treatment during the both years of experimentation. However, the seed protein contents were higher where no growth regulator was applied (Table 3).

dry matter yield. Better stay green eventually results in better assimilates translocation during the reproductive and grain filling stages thus improving the number of silique per plant (Table 2, 3; Khorshidi *et al.*, 2013), seeds per silique (Table 3; Khorshidi *et al.*, 2013), and 1000-seed weight

Table 3: Influence of nitrogen application at variable rates in combination with plant growth regulators on morphological/yield parameters, seed yield, seed oil contents and seed protein contents of canola

	No. of seeds per silique		1000-seed weight (g)		Seed yield (kg ha ⁻¹)		Seed oil contents (%)		Seed protein contents (%)	
	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16
Nitrogen levels (N; Kg ha ⁻¹)										
100	26.9AB	28.3AB	3.50A	3.60	2031	2101A	34.8	33.9	19.6	20.8
60	26.6BC	27.6B	3.28B	3.92	2047	2072B	34.5	34.0	19.2	20.4
80	25.8C	26.4C	3.53A	3.91	2085	2077B	35.1	34.0	19.5	20.9
120	27.7A	29.3A	3.51A	4.05	2056	2110A	35.5	34.1	19.8	21.5
LSD ($p \leq 0.05$)	1.02	0.54	0.18	NS	NS	20.0	NS	NS	NS	NS
Plant growth regulators (PGR; 100 ppm)										
Control	25.1D	26.5C	2.90D	3.08B	1868D	1953D	30.5D	29.8D	22.8A	24.4A
SA	26.2C	27.1C	3.37C	3.81AB	2037C	2058C	33.2C	32.5C	21.2B	22.4B
GA	27.2B	28.3B	3.63B	4.02AB	2120B	2141B	36.5B	35.3B	18.9C	20.1C
SA+GA	28.5A	29.7A	3.92A	4.56A	2197A	2213A	39.6A	38.4A	15.7D	16.7D
LSD ($p \leq 0.05$)	0.70	1.11	0.15	0.46	33.0	36.7	0.08	0.72	0.69	0.73
PGR×N	NS	1.09	0.30	NS	NS	NS	0.17	NS	NS	NS

Means of nitrogen and plant growth regulators sharing the same letter, do not differ significantly at $p \leq 0.05$; SA= Salicylic acid; GA= Gibberellic acid; NS= non-significant.

Discussion

The response of oilseed crops to soil applied N fertilizer is positive even at high rates as it is required for harvesting the maximum productivity (Stahl *et al.*, 2017; Gu *et al.*, 2018). As the oilseed crops are grown on marginal lands (deficient in macro and micronutrients) under semi-arid and arid environments, the N application becomes vital to harvest better seed yields. The nitrogen requirement of canola is higher than the other oilseed crops and considered as a high N demanding crop (Rathke *et al.*, 2005; Pan *et al.*, 2016; Ma and Zheng *et al.*, 2016). Thus, it is critical to optimize the N application to exploit the full potential of canola crop under arid and semi-arid regions.

In the current study, N application significantly influenced some of the yield parameters (seeds per silique and siliques per plant) of canola; and they were the maximum when N was applied at 120 or 100 kg ha⁻¹ than that of N application at low rates (60 and 80 kg ha⁻¹). Indeed, N is required for protein synthesis, leaf development, flower/fruit development, and is a part of chlorophyll (Kumar *et al.*, 2018; Saini *et al.*, 2017; Ul-Allah *et al.*, 2015) thus imparts the stay green and finally improves the vegetative growth of crops. This improvement in vegetative growth of the plants ultimately increases the

(Table 3), as the same was observed in this study. In oilseed crops, the 1000-seed weight, seeds per silique and silique per plant are the main yield contributing traits. Thus, the improvement in these yield parameters due to application of N at higher rates resulted in highest seed yield. Low seed yield at the lower N application rates might be attributed to lower 1000-seed weight, silique per plant, seeds per silique (Tables 2, 3).

Beside macronutrients, PGRs (e.g., salicylic acid, cytokinin, gibberellins and abscisic acid) helps the plants to perform better under optimal and suboptimal conditions (Farooq *et al.*, 2009; Khan *et al.*, 2015; Siddiqui *et al.*, 2016) than the plants not supplied with these PGRs. On the other hand, gibberellic acid is an important PGR that may improve the growth cascades of plants by stimulating the stem and internode elongation, enzyme activation, and the fruit setting (Colebrook *et al.*, 2014; Dar *et al.*, 2015), thus improving the yield parameters. Indeed, the PGRs divert the photosynthate towards the harvested product thus enhancing the actual productivity (Table 3; Abdelgadira *et al.*, 2010). Seemingly, the combined foliage application of both PGRs improved the allometric and morphological parameters which resulted in better seed formation which was visible through improvement in 1000-seed weight and seeds per

silique (Hayat *et al.*, 2007; Mijic *et al.*, 2006). Better yield parameters thus resulted in better seed yield in this study (Table 3).

Although N application did not significantly affect the seed oil and seed protein contents, the combined application of both PGRs significantly enhanced the seed oil contents and reduced the seed protein contents. Improvement in seed oil composition can be attributed to the activation of the synthesis of various enzymes which are involved in the metabolism of fatty acids (Talaat and EL-Din, 2007; Colebrook *et al.*, 2014; Dar *et al.*, 2015), thus enhancing seed oil contents as was observed in this study. However, seed protein contents were decreased due to sole or combined application of PGRs. Indeed, the oil contents follow reverse order against the protein content in most oilseed crops (Bellaloui *et al.*, 2015). As, the oil contents were enhanced due to exogenous application of PGRs, thus the protein contents were decreased.

Conclusion

In conclusion, combined application of gibberellic acid and salicylic acid enhanced the morphological and yield parameters which eventually enhanced the seed yield, seed oil contents and decreased the seed protein contents. Application of N at higher rates was most beneficial for improvement in yield parameters and seed yield than its application at low levels. The interactive effect showed that significantly higher number of silique per plant, number of seeds per silique and seed yield were recorded with combined application of gibberellic acid and salicylic acid with N application @ 120 kg ha⁻¹.

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