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Effect of sowing method, nitrogen and GABA on growth dynamics of maize (*Zea mays* L.) under excess soil moisture stress

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Abstract
Excess soil moisture stress is one of the major constraints in maize growth and development. Proper crop management practices need to be developed to improve growth of plant under excess soil moisture stress. Therefore, a field experiment was conducted for two years during kharif season 2019 and 2021 at G.B. Pant University of Agriculture and Technology, Pantnagar, to observe the influence of sowing method, amount of nitrogen and foliar application of gamma amino butyric acid (GABA) on excess soil moisture stressed maize. Six treatment combinations of nitrogen and GABA {recommended dose of N (RDN), RDN + 30 kg N/ha, RDN + 1 mM GABA, RDN + 2 mM GABA, RDN + 30 kg N/ha + 1 mM GABA and RDN + 30 kg N/ha + 2 mM GABA} were tested under two sowing methods *viz*., flat and ridge. Thus, twelve treatments were analyzed in factorial RBD with three replications. The recommended dose of nutrients was 120:60:40 kg N: P_2O_5:K_2O/ha. Artificial ponding was created at knee height stage continuously for 7-days. The depth of standing water was maintained 5 cm in the field. Extra 30 kg N was top dressed 4 days after drainage of water and foliar application of GABA was made thrice 3, 10 and 17, four days after draining of ponded water. Nitrogen and GABA had significant positive impact on growth of plants. Stressed plants treated with RDN+ 30 kg N + 2 mm GABA exhibited the highest AGR, CGR and RGR. Maize grown on ridge attained significantly 7.6% more AGR (2.82 g/plant/day) and 13.4% CGR more (1.27 g/cm^2/day) and 2.2% higher RGR (18.5 mg/g/day) at 51- 65 DAS than flat sowing. Maximum AGR, CGR and RGR were obtained with RDN + 30 kg N + 2 mM GABA application. Results revealed that adverse effects of excess soil moisture stress on growth of maize plants can be overcome by growing on ridge and applying extra 30 kg N/ha.

Keywords: Sowing method, Nitrogen, GABA, AGR, CGR, RGR and excess soil moisture, maize

1. Introduction
Maize (*Zea mays* L.) is one of the most versatile emerging crops, having greater adaptability under varied agro-climatic conditions. It is not only an important food crop, but also a broad source of feed, fodder, and raw material for the manufacturing of many industrial products. Maize can be used for medical purpose like maize silk is used for bladder infection, inflammation of the urinary system, inflammation of the prostate kidney stone and bedwetting (Hasanudin *et al*., 2012) [4]. Maize is considered the third most important food crop among the cereals in India and contributes to nearly 9 per cent of the national food basket. The consumption pattern of maize (feed-64%, food-16%, industry-19%, and seed and other miscellaneous 1%) in India largely matches that of the global pattern (feed-61%, food-17%, and industry-22%) (Malhotra, 2017) [6]. In terms of area and production, maize is the third most important staple food crop in the world after wheat and rice, but in terms of productivity, it ranks first followed by rice and wheat. Worldwide, maize is cultivated on approximately 197 million ha area with a production of 1,144.63 million tonnes and a productivity of 5.81 t/ha. The United States of America (USA) is the largest producer of maize, contributing nearly 35 per cent of the total production in the world. (Foreign Agricultural Service/USDA, 2020-21) [3]. In India, maize is cultivated on a 9.2 million ha area, with production and productivity of 28 million tonnes and 3.0 t/ha, respectively. (Director's review, IIMR, 2020-21) [2]. Excess soil moisture stress caused by temporary waterlogging or poor drainage conditions is one of the major constraints for maize production not only in India, but in the entire Asian region (Laurentius and Julia, 2015) [5]. The adverse effect of excess soil moisture or waterlogging on crop may be curtailed by adopting proper planting method. Commonly, maize is grown on the flat beds.

There is no drainage system in this planting method, which causes leaching and creates anaerobic condition in rhizosphere. Maize plants grown by flat method have restricted growth under waterlogged condition (Ren *et al*., 2018) [10]. Plant tolerance to waterlogging stress may be increased by nitrogen fertilizer application nitrogen may improve plant adaptative mechanisms to waterlogging, such as root growth and adventitious root regrowth after excess soil moisture stress. Gamma aminobutyric acid (GABA) is a significant component in most prokaryotic and eukaryotic organism in hypoxia condition. It is consist of four-carbon non-protein genic amino acid. It plays a dual role in regulating carbon–nitrogen balance and nitrogen metabolism. It is also involved in response to many aspects of physiological metabolism, such as antioxidant capacity under waterlogging (Ramesh *et al*., 2015) [9]. Crop yield is shown to be correlated with physiological growth indices such as net assimilation rate (NAR), crop growth rate (CGR), leaf area index (LAI) and relative growth rate (RGR) (Ruchi, 2008) [11]. The objective of this experiment was to study the effects of sowing method, nitrogen and growth regulator on growth of maize grown under excess soil moisture. This study will be useful for providing theoretical and technical support for the development of effective water-tolerance cultivation process.

2. Materials and Methods

A field experiment was conducted during the *kharif* season 2019 and 2021 at G.B. Pant University of Agriculture and Technology, Pantnagar, This region is *Tarai* which is developed from medium to moderately coarse textured calcareous parent material under the predominant influence of forest vegetation with moderately to poor drained conditions. These soils are originated from alluvial sediments and belong to soil order mollisols. The climate of Pantnagar is humid subtropical. During summers conditions are hot and dry and winters are harsh. Six treatment combinations of nitrogen and GABA {recommended dose of N (RDN), RDN + 30 kg N/ha, RDN +1 mM GABA, RDN +2 mM GABA, RDN + 30 kg N/ha + 1 mM GABA and RDN + 30 kg N/ha + 2 mM GABA} were tested under two sowing methods *viz*., flat and ridge (ridge height maintain 10-12cm apart from row). Prior to sowing, the field site was ploughed three times approximately 30 cm deep using a cultivators and rotavatrs to destroy off types. Fine seed beds were prepared before sowing. The maize variety "DKC1944" was sown at a spacing 60×25 cm between row and plants. Thus, twelve treatments were analyzed in factorial RBD with three replications. The recommended dose of nutrients was 120:60:40 kg N: P2O5:K2O/ha. Artificial ponding was created at knee height stage continuously for 7-days. The depth of standing water was maintained 5 cm in the field. Extra 30 kg N was top dressed and foliar application of GABA was made thrice 3, 10 and 17, four days after draining of ponded water.

3. Statistical Analysis

The data obtained from various observations will be statistically analyzed as per procedure of factorial randomized block design by using the standard techniques of Analysis of Variance (ANOVA) was used to with the help of computer software programme using OP STAT. If "F" test will be found significant at 5% level of significance (p<0.05) the critical difference (C.D) will be calculated to test the significance of differences between two treatment means.

4. Growth analysis

Following growth analysis parameters were computed by using formula as reported by Radford (1967) [8].

4.1 Absolute Growth Rate (AGR): It represents dry weight gained per unit time. It was calculated as:

$$\text{Absolute growth rate} = \frac{W2-W1}{t2-t1}$$

Where,
W_1 and W_2 are dry weight of whole plant at time t_1 and t_2, respectively
It is expressed in g/plan/day

4.2 Crop growth rate

It represents dry weight gained per unit time in a unit ground area. It was calculated as:

$$\text{Crop growth rate} = \frac{W2-W1}{t2-t1}\times 1/\text{plant geometry}$$

W_1 and W_2 are dry matter production at time t_1 and t_2, respectively in a unit area
t_1 and t_2 are days of first and second sampling, respectively
It is represented in g/cm^2/day.

4.3 Relative growth rate

It represents increase in dry matter production per unit dry matter already exist in a unit time in a unit area. It was be calculated with the help of following formula:

$$\text{Relative growth rate} = \frac{lnw2-lnw1}{t2-t1}$$

W_1 and W_2 are dry matter production at time t_1 and t_2, respectively in a unit area.
It is expressed as g/g/day.

5. Results and Discussion

5.1 Absolute growth stage (AGR)

AGR increased with the advancement of crop age and reached maximum at growth interval 51 to 65 DAS (Table 1). Both the sowing method (ridge and flatbed) in excess soil moisture condition did not exhibit significant difference in AGR at 0-30 DAS growth period during both the years. However, numerically more values of AGR (0.51 and 0.27 g/plant/day) were observed in ridge than flatbed during both the years. AGR varied significantly by sowing method at 30-51 DAS, 51-65 DAS and 65 DAS to harvest stage during both the years, where ridge sowing recorded significant higher absolute growth rate than flatbed sowing. The increase in absolute growth rate per plant under ridge sowing 8, 7.6 and 21.4 per cent more on at 30-51 DAS, 51-65 DAS and 65 to harvest, respectively mean basis. An increase in AGR was noticed with increase in nitrogen and GABA dose. But was not affected significantly at 0- 30 DAS. At growth stage interval 30-51 DAS, RDN+30 kg N + 2 mM GABA combination showed significantly higher value (1.81 and 1.43 g/day in 2019 and 2021 than other treatments. But remained at par with RDN+30 kg N and RDN+30 kg N + 1 mM GABA during both the years. At 51-65 DAS RDN+30 kg N + 2 mM GABA showed significantly higher value (4.0 g/plant/day) in 2019 than RDN and remained at par with rest of the treatment. In 2021 RDN+30 kg N showed significantly higher value (1.95 g/plant/day) but did not vary statistically with RDN+30 kg N + 2 mM GABA and RDN+30 kg N + 1 mM GABA. While, 65 DAS to harvest stage, RDN+ 30kg N + 2 mM GABA combination showed significantly higher value (3.68 and 1.78 g/plant/day in 2019 and 2021, respectively) than other treatments but remained at par with RDN+30 kg N and RDN+30 kg N + 1 mM GABA combination.

Since maize plant is highly sensitive to waterlogging hence excess moisture stress caused lower metabolic processes of plant and reduced the dry matter production. It led to causing less vegetative growth. At different crop growth stages variation in absolute growth rate were observed because of differences in dry matter production per plant higher dry matter production was responsible for more value of AGR. Less dry matter production per plant which is related with low absolute growth rate and similar results were also found by Amanullah *et al.* (2010) [1].

Plant grown on ridge faced better aerobic condition in rhizosphere which helped in absorption of nutrient and moisture. It resulted into more dry matter production Ruchi, (2008) [11].

Table 1: Effect of planting method, nitrogen and GABA application on absolute growth rate in maize under excess soil moisture condition

Treatment	AGR (g/plant/day)											
	0-30 DAS			30-51 DAS			51-65 DAS			65 DAS to Harvest		
	2019	2021	Mean	2019	2021	Mean	2019	2021	Mean	2019	2021	Mean
Planting Method												
Ridge	0.51	0.27	0.39	1.61	1.36	1.49	3.82	1.81	2.82	2.86	1.56	2.21
Flat bed	0.49	0.26	0.38	1.47	1.29	1.38	3.54	1.70	2.62	2.27	1.37	1.82
SEm ±	0.006	0.007		0.04	0.02		0.08	0.04		0.17	0.06	
CD (P=0.05)	NS	NS		0.11	0.05		0.25	0.12		0.51	0.18	
RDN	0.48	0.24	0.36	1.20	1.19	1.20	2.56	1.40	1.98	1.55	1.02	1.29
RDN + 30 kg N	0.50	0.26	0.38	1.71	1.38	1.55	3.9	1.95	2.93	2.98	1.55	2.27
RDN + 1 mM GABA	0.49	0.26	0.38	1.41	1.27	1.34	3.76	1.65	2.71	1.98	1.38	1.68
RDN + 2 mM GABA	0.50	0.27	0.39	1.45	1.31	1.38	3.95	1.66	2.81	1.95	1.37	1.66
RDN + 30 kg N + 1mM GABA	0.51	0.28	0.40	1.68	1.38	1.53	3.90	1.92	2.91	3.28	1.70	2.49
RDN + 30 kg N + 2 mM GABA	0.53	0.29	0.41	1.81	1.43	1.62	4.0	1.93	2.97	3.68	1.78	2.73
S.Em ±	0.011	0.011		0.06	0.03		0.15	0.03		0.30	0.10	
CD (P=0.05)	NS	NS		0.20	0.08		0.44	0.10		0.90	0.31	

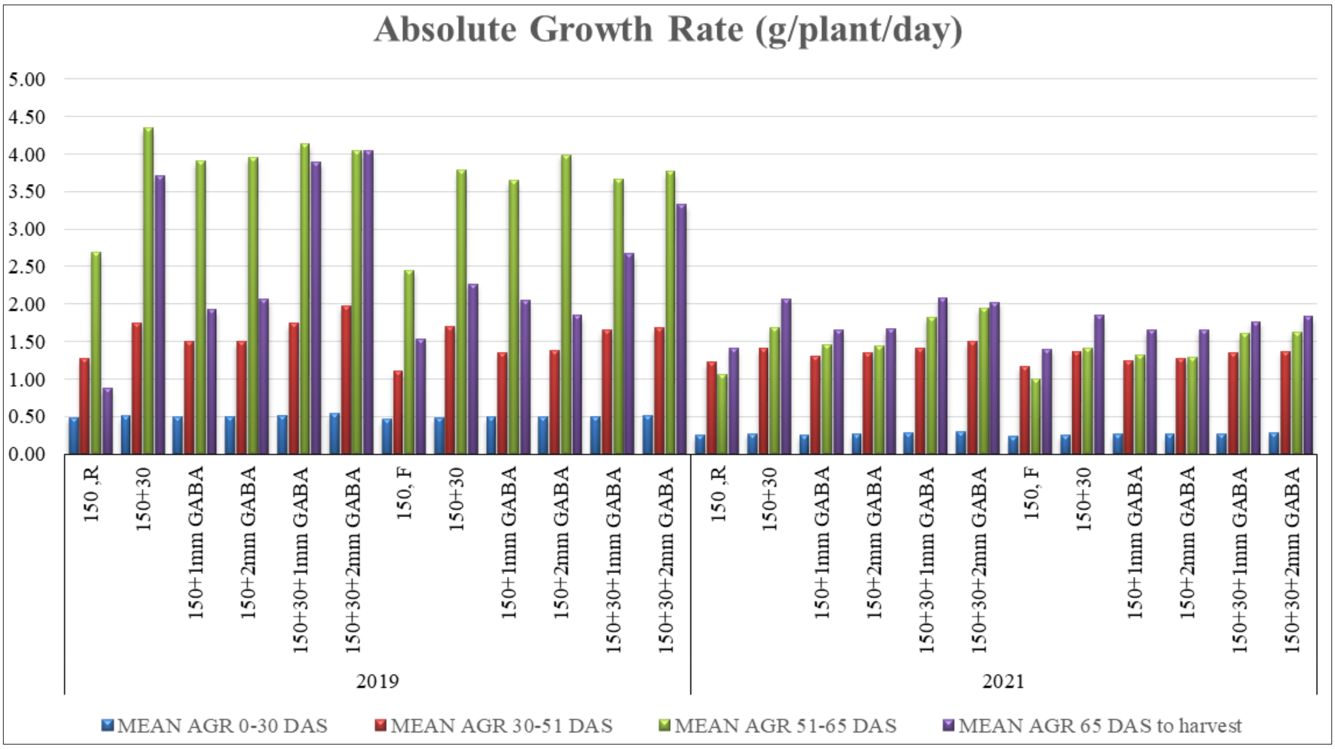

Fig 1: Effect of sowing method, Nitrogen and GABA on absolute growth rate of maize under excess soil moisture stress.

5.2 Crop growth rate (CGR)

The data recorded for crop growth rate (Table 2) showed that CGR increased with the advancement of crop age and reached maximum at growth interval 51-65 DAS.

Non-significant difference in CGR at 0-30 DAS growth period between both the sowing methods were observed during both the years. However, numerically more values of CGR (0.21 and 0.12 g/cm^2/day) were noted in ridge method. at 30-51 DAS, 51-65 and 65 DAS to harvest growth interval significant differences in CGR were recorded while ridge sown crop attained significantly more value of CGR (0.67 and 0.57, 1.59 and 0.95 and 1.19 and 0.65 g/cm^2/day) than flatbed sown crop. CGR was increased by application of nitrogen and GABA. But was not affected significantly at 0- 30 DAS. At growth stage interval 30-51 DAS, RDN + 30 kg N + 2mM GABA had significantly higher CGR (0.75 g/cm^2/day in 2019 and 0.60 g/cm^2/day in 2021) but remained at par with RDN+30 kg N and RDN+30 kg N + 1 mM GABA. at 51-65 DAS where RDN+30 kg N showed significantly higher value (1.69 g/cm^2/day) in 2019 being at par with RDN+30 kg N + 1 mM GABA and RDN +30 kg N + 2 mM GABA. But in 2021 RDN+30 kg N + 2 mM GABA exhibited significantly higher value (0.95 g/cm^2/day). But and remained at par with RDN + 30 kg N and RDN+30 kg N + 1 mM GABA. At 65 DAS to harvest stage RDN+ 30 kg N + 2 mM GABA also showed significantly higher CGR (1.54 g/cm^2/day in 2019) than other treatments but did not vary statistically with RDN + 30 kg N and RDN+30 kg N + 1 mM GABA. Moreover this treatment in 2021 which was at par with all treatment except RDN.

CGR depends on rate dry of matter accumulation per day in a unit area differences in CGR was due to differential growth rate under excess soil moisture condition. Higher value of CGR under GABA and nitrogen application treatment and in ridge sown crop was due to more dry matter production. Similar results were reported by Yasari and Patwardhan (2006) [12].

Table 2: Effect of planting method, nitrogen and GABA application on crop growth rate in maize under excess soil moisture condition

Treatment	CGR (g/cm^2/day)											
	0-30 DAS			30-51 DAS			51-65 DAS			65 DAS to Harvest		
	2019	2021	Mean	2019	2021	Mean	2019	2021	Mean	2019	2021	Mean
Planting method												
Ridge	0.21	0.12	0.17	0.67	0.57	0.62	1.59	0.95	1.27	1.19	0.65	0.92
Flat bed	0.20	0.11	0.16	0.61	0.54	0.58	1.48	0.75	1.12	0.95	0.57	0.76
SEm ±	0.003	0.003		0.01	0.007		0.02	0.02		0.08	0.03	
CD (P=0.05)	NS	NS		0.04	0.02		0.05	0.06		0.23	0.09	
Nitrogen and GABA application												
RDN	0.19	0.10	0.15	0.50	0.50	0.50	1.07	0.69	0.88	0.65	0.43	0.54
RDN + 30 kg N	0.21	0.11	0.16	0.71	0.58	0.65	1.69	0.90	1.30	1.24	0.64	0.94
RDN + 1 mM GABA	0.20	0.11	0.16	0.59	0.53	0.56	1.57	0.82	1.20	0.82	0.58	0.70
RDN + 2 mM GABA	0.21	0.11	0.16	0.60	0.54	0.57	1.65	0.82	1.24	0.81	0.57	0.69
RDN + 30 kg N + 1mM GABA	0.21	0.12	0.17	0.70	0.57	0.64	1.61	0.92	1.27	1.37	0.71	1.04
RDN + 30 kg N + 2 mM GABA	0.22	0.13	0.18	0.75	0.60	0.68	1.63	0.95	1.29	1.54	0.74	1.14
SEm ±	0.005	0.005		0.02	0.01		0.03	0.04		0.13	0.06	
CD (P=0.05)	NS	NS		0.07	0.03		0.08	0.11		0.40	0.18	

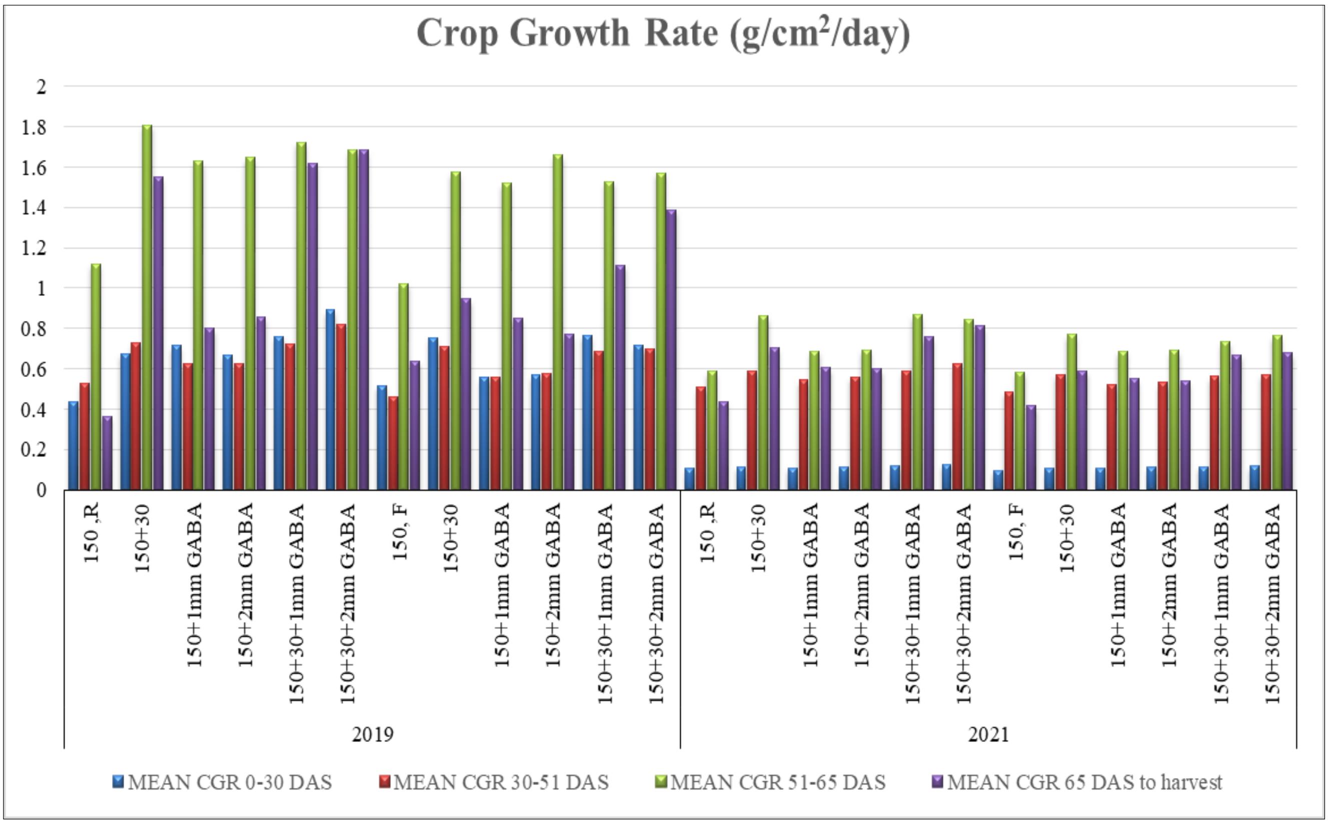

Fig 2: Effect of sowing method, Nitrogen and GABA on crop growth rate of maize under excess soil moisture stress.

5.3 Relative growth rate (RGR)

The data pertaining to relative growth rate (RGR) as given in Table 3 indicated that it decreased with advancement in crop age.

Differences in RGR in relation to sowing method (ridge and flatbed) were found non-significant during all the stages of crop growth during both the years. However, numerically more value of RGR was recorded in ridge method at 30-51

DAS (24.2 and 31.2 mg/g/day), 51-65 DAS (22.8 and 14.2 mg/g/day) and 65 DAS to harvest (4.8 and 8.8 mg/g/day) during both the years. An increase in RGR was noticed with increase in nitrogen and GABA dose. But RGR did not reach to the level of significance by nitrogen and GABA application at all growth stage interval. However, numerically more value of RGR observed at 30-45 DAS (25.5 and 32.0 mg/g/day) and 51- 65 DAS (25.2 and 15.3 mg/g/day) and 65 to harvest stage (5.6 and 9.2 mg/g/day) in RDN +30 kg N during both the years.

RGR depends on rate of dry matter accumulation per unit existing dry matter in unit time. The present study also indicated that those plant treated with GABA and nitrogen acquired maximum dry matter accumulation possibly due to both beneficial factor in which GABA could alleviate the excess soil moisture stress due to increase antioxidant capacity and nitrogen could enhance growth of plant by increasing carbon and nitrogen metabolism. Decrease in relative growth rate due to waterlogging stress has been reported in many crops (Qasim *et al*., 2004) [7].

Table 3: Effect of planting method, nitrogen and GABA application on relative growth rate (RGR) of maize at different growth intervals under excess soil moisture condition

Treatment	RGR (mg/g/day)								
	30-51 DAS			51-65 DAS			65 DAS to Harvest		
	2019	2021	Mean	2019	2021	Mean	2019	2021	Mean
Planting method									
Ridge	24.2	31.2	27.7	22.8	14.2	18.5	4.8	8.8	6.8
Flat bed	23.3	30.0	26.7	22.7	13.5	18.1	4.4	8.7	6.6
S.Em ±	0.6	0.6		0.7	0.7		0.3	0.4	
CD (P=0.05)	NS	NS		NS	NS		NS	NS	
Nitrogen and GABA application									
RDN	20.8	30.0	25.4	20.2	11.2	15.7	4.0	8.5	6.3
RDN + 30 kg N	25.5	32.0	28.8	25.2	15.3	20.3	5.6	9.2	7.4
RDN + 1 mM GABA	22.7	30.7	26.7	22.3	13.8	18.1	3.8	8.6	6.2
RDN + 2 mM GABA	22.8	30.8	26.8	22.8	13.3	18.1	3.8	8.6	6.2
RDN + 30 kg N + 1mM GABA	24.9	31.0	28.0	23.5	14.0	18.8	5.3	8.8	7.1
RDN + 30 kg N + 2 mM GABA	25.4	31.0	28.2	24.0	15.3	19.7	5.5	8.6	7.1
S.Em ±	1.2	1.0		1.2	1.2		0.6	0.7	
CD (P=0.05)	NS	NS		NS	NS		NS	NS	

Conclusion

Experimental finding indicated that nitrogen and GABA have pronounced effect on alleviating excess soil moisture stress in maize but combined application of GABA and nitrogen have potential to overcome the losses due to excess soil moisture stress. Growth of maize under temporary waterlogged condition can be maintained by growing it on ridges.

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