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Sai Meghana Palacharla
PG Scholar, Department of Soil
Science, Professor Jayashankar
Telangana Agricultural University,
Hyderabad, Telangana, India

A Aziz Qureshi
PG Scholar, Department of Soil
Science, Professor Jayashankar
Telangana Agricultural University,
Hyderabad, Telangana, India

P Kadirvel
PG Scholar, Department of Soil
Science, Professor Jayashankar
Telangana Agricultural University,
Hyderabad, Telangana, India

K Shailaja
PG Scholar, Department of Soil
Science, Professor Jayashankar
Telangana Agricultural University,
Hyderabad, Telangana, India

Impact of variation in kernel nitrogen content on the growth performance of seedlings in a safflower core germplasm under different nitrogen levels

Sai Meghana Palacharla, A Aziz Qureshi, P Kadirvel and K Shailaja

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Abstract

Kernel nitrogen is an important quality parameter that plays a crucial role in seedling establishment and early plant growth before active nutrient uptake from the soil begins. Most studies on safflower have focused on the effects of applied nitrogen fertiliser on growth and yield parameters. However, studies on the specific role of kernel nitrogen reserves in influencing germination, vigour, and seedling establishment under varying nitrogen availability during early seedling development have not been reported. A paper towel study was conducted to evaluate the growth of seedling with variation in kernel nitrogen content. For which, from the safflower core consisting 100 genotypes, 10 genotypes with high kernel nitrogen content were selected along with a check CGH-69 (with lowest kernel N content) for comparative evaluation under three nitrogen levels: 0N (N0), 50%N (N1), and 100%N (N2) prepared with Hoagland solution. Observations were recorded for shoot length, root length, germination percentage, seedling vigour and dry weight. Significant variation was observed among the genotypes for all the parameters studied. Increasing the nitrogen level from N0 to N2 significantly improved seedling growth across all genotypes. Among the genotypes, CGH-41 recorded superior shoot length (11.2 cm seedling⁻¹) and root length (21.87cm seedling⁻¹). CGH-72 showed the highest germination percentage (95.6%). CGH-278 exhibited superior seedling vigour (55.5g seedling⁻¹) and dry matter (0.6g seedling⁻¹ whereas the check genotype CGH-69 recorded the lowest shoot length (6.29cm seedling⁻¹) and root length (14.59 cm seedling⁻¹) germination percentage (78.9%), seedling vigour (14.2g seedling⁻¹) and dry weight (0.18 g seedling⁻¹) respectively. The observed genotypic variation provides valuable information for identifying nitrogen-efficient safflower genotypes.

Keywords: Kernel nitrogen, vigour, germination percentage

1. Introduction

Safflower is a minor oilseed crop belonging to the family *Asteraceae* and originating from the Middle East. A distinctive feature of safflower is its adaptation to hot, dry regions, supported by a taproot system that requires less water. It is mainly grown for its superior seed oil, which is rich in unsaturated fatty acids. Safflower is also an excellent forage crop due to its high quality (Bartal *et al.*, 2008) [5], and its petal are used as a natural food colouring agent (Steberl *et al.*, 2020) [18]. Favourable growth conditions for safflower typically occur during the winter season (Rabi), from October/November to March/April. Among the seven edible oils cultivated in India, safflower accounts for less than 3.71% of the production share. Maharashtra leads with the highest output of 0.26 lakh tonnes, followed by Karnataka with 0.14 lakh tonnes and Telangana with 0.03 lakh tonnes, together accounting for more than 90% of India's production. The area, production and productivity of safflower in India are 0.56lakh ha, 0.43 lakh tonnes, and 640kg ha⁻¹, respectively.

Nitrogen is an essential macronutrient that plays an important role in plant growth and development. It is a main component of proteins and enzymes in leaves and acts as a functional system for photosynthesis, thereby influencing crop productivity. Seed nitrogen is a decisive factor during the initial stages of plant development. Proteins stored in the seeds serve as a major source of nitrogen during germination and seedling establishment. Before the root system becomes functional, the developing seedling relies on the nitrogen reserves stored in the seed to support cell division, tissue formation, and early growth. Many studies have reported that higher

Corresponding Author:
Sai Meghana Palacharla
PG Scholar, Department of Soil
Science, Professor Jayashankar
Telangana Agricultural University,
Hyderabad, Telangana, India

seed nitrogen is associated with increased seedling vigour when soil nutrients are low (Bulsani and Warner, 1980, Hara and Toriyama, 1998, Nedel *et al.*, 1996; Parrish and Bazzaz, 1985; Ries, 1971, Tungate *et al.*, 2002; Welch, 1999) [6, 12, 13, 15, 16, 19, 20].

2. Materials and Methods

2.1 Location of the study

The proposed paper towel experiment was conducted at Soil Science Laboratory, in ICAR-Indian Institute of Oilseed Research, Hyderabad, Telangana, India.

2.2 Estimation of Seed nitrogen content in 100 safflower germplasm

The seeds were allowed to soak in water overnight, and the hull was carefully removed. After drying at 70°C until constant weight, the N content was estimated in the Kernels. Kjeldahl digestion and distillation method was adopted as described by Jackson (1974). A 0.5 g of finely powdered kernel sample was weighed into a 250 mL digestion tube. For digestion, a 10 mL of conc. H₂SO₄ and 3-4 g of catalyst mixture (K₂SO₄ + CuSO₄ in a ratio of 5:1) were added to the tubes were placed in a Kelplus digestion unit and heated at 410°C for 3 hours till the digestion was completed by observing clear blue solution in the tubes. The test tubes were removed from the digestion chamber and cooled to room temperature. Each tube with the digested content was placed in the distillation unit. To a 250ml conical flask, 20 ml of 4% boric acid containing mixed indicator was taken in a 250 ml conical flask and placed under the receiver tube (dipped the receiver tube end in the boric acid). Then, 15 mL of 40% NaOH was added to the digestion tube, and the distillation process (running steam) was carried out for 6 minutes. After 6 minutes of distillation, the conical flask was removed and titrated with 0.02 N H₂SO₄ until the colour changed from bluish green to wine red. Nitrogen content was calculated from the titre value and expressed as percent dry weight of the kernel.

2.3 Paper towel experiment:

Ten safflower genotypes along with one check were selected for the paper towel experiment based on high kernel nitrogen content CGH-41(0.72%), CGH-85(0.64%), CGH-72 (0.61%) CGH-16(0.60%), CGH-118(0.57%), CGH-128(0.55%), CGH-128, (0.55%), CGH-13(0.52%) CGH-219(0.49%), CGH-104(0.44%) and check CGH-69 (0.25%).

2.4 Preparation of Hoagland's solution

The composition of macronutrient stock solution included: calcium nitrate (5.903g), magnesium sulphate (2.46g), and potassium dihydrogen phosphate (1.36g) dissolved in 100ml of double distilled water. The micronutrients stock solution comprised of boric acid (1.43g), manganese chloride (0.915g), zinc sulphate (0.11g), copper sulphate (0.025g), and sodium molybdate (0.06g) dissolved in 500ml of distilled water. The Fe-EDTA stock solution was prepared separately by mixing the required quantity of EDTA (5.2g) and ferrous sulphate (3.9g) in 500ml of distilled water. For the half (N1) and full (N2) strength nitrogen levels 5 mL and 10 mL of calcium nitrate stock solution are mixed in 1 L of water, respectively. Remaining nutrients are mixed constantly in 1 L of water in all the treatments (Arnon, D.I and Hoagland, 1950) [4].

2.4 Paper towel experiment for germination and vigour

For this laboratory experiment, brown germination papers were moistened uniformly with distilled water, and the clean seeds were placed evenly on the top of the paper. Another wet germination paper was placed over the seeds. The double sheet

was then folded at 1inch at the base and the sheets with the seeds were rolled carefully from one side to form a compact roll. Then each roll was labelled with the genotype number, treatment, and replication number, and secured with rubber bands at both ends. The rolls were placed with seed position upright in beakers containing Hoagland nutrient solution as per the treatment. The paper towels were maintained under suitable conditions for germination and seedling growth. After 10 days the paper towel was opened and recorded the following Observations such as root and shoot length, germination percentage, dry weight and the seedling vigour.

2.5 Statistical analysis

The data generated in the paper towel experiment was statistically analysed by adopting 2-factor CRD. Factor -1: 11 Genotypes and factor-2: 3 Nitrogen levels.

3 Results and Discussion

A set of 100 genotypes from safflower core was characterized for kernel nitrogen content. From the core, the 10 genotypes having high kernel nitrogen content along with check were selected for further studies to assess the germination and vigour test in a laboratory experiment adopting paper towel experiment.

3.1 Effect of different levels of nitrogen on growth parameters of the seedlings

3.1.1 shoot length

Among the genotypes, the shoot length values showed variation at the 10th day of the germination. The genotype CGH- 41 recorded the highest shoot length (11.2 cm) followed by CGH-278 (10.6 cm), CGH-85 (10.4 cm) and the lowest shoot length was recorded in check, CGH-69 (6.2 cm). Genotypes also showed variable response in shoot length with the application of external nitrogen levels. The highest mean shoot length was recorded due to nitrogen level N2 (10.5 cm), followed by N1 (9.6 cm) and N0 (8.3 cm) as shown in Table 1. The interaction effect of genotype and N levels on shoot length was found to be prominent. The genotype CGH-41 recorded the highest shoot length of 11.8cm due to N2 level of N application. This was followed by CGH-72 (11.7cm) with N2 level. However, at N0 level of N, genotype CGH-41 produced the highest shoot length of 10.4cm against check CGH-69 (5.4cm). The improved shoot growth may be due to increased nitrogen availability and genotypic differences in nitrogen utilization, promoting greater cell division and elongation leading to greater shoot growth. Similar findings were recorded by Slot *et al* (2013) [17] and Erbaş *et al* (2016) [9].

Table 1: Effect of different nitrogen levels on seedling shoot length (cm seedling⁻¹)

Genotype	N0	N1	N2	MEAN
CGH-219	7.5	10.1	10.3	9.3
CGH-278	10.2	10.4	11.4	10.7
CGH-72	7.1	10.6	11.7	9.8
CGH-13	9.2	9.7	10.8	9.9
CGH-41	10.4	11.5	11.8	11.2
CGH-16	9.7	10.2	11.2	10.3
CGH-85	9.3	10.5	11.5	10.4
CGH-118	8.8	9.8	11.5	10.0
CGH-128	8.1	9.5	10.1	9.3
CGH-104	6.1	7.6	8.4	7.4
CGH-69(check)	5.4	6.3	7.2	6.3
MEAN	8.3	9.7	10.6	
	(A)	(B)	(AXB)	
SE(m)	0.10	0.05	0.17	
CD	0.28	0.14	0.25	

3.1.2 Root Length

Among the genotypes, the root length values showed variation at the 10th day of the germination. The genotype CGH- 41 recorded the highest root length (21.8 cm) followed by CGH-16 (19.8 cm), CGH-85 (19.1 cm) and the lowest root length was recorded in CGH-69 (14.5 cm). Genotypes also showed variable response in root length with the application of external nitrogen levels. The highest root length was recorded due to N2 level (20 cm), followed by N1 (17.6 cm) and N0 (15.3 cm) as shown in the Table 2. The interaction effect of genotype and N levels on root length was found to be prominent. The genotype CGH-41 was recorded the highest root length of 23.3 cm due to N2 level of N application. This was followed by the genotype CGH-16 (20.6 cm) with the N2 level. However at N0 level of N application the genotype CGH-41 produced the highest root length of 20.3cm against the check (12.5cm). The positive response for root length growth between genotypes to the nitrogen was recorded. Further, genotypes with high seed nitrogen content showed better root growth. Likewise, similar findings were reported by Abbadi *et al.* (2008) ^[1], Abdurahman *et al.* (1999) ^[2].

Table 2: Effect of different nitrogen levels on seedling root length (cm seedling⁻¹)

Genotype	N0	N1	N2	Mean
CGH-219	12.5	16.6	19.2	16.1
CGH-278	14.5	18.6	20.6	17.9
CGH-72	11.4	16.2	19.8	15.8
CGH-13	16.2	18.5	20.5	18.4
CGH-41	20.3	21.5	23.7	21.8
CGH-16	18.8	20.1	20.6	19.8
CGH-85	18.2	18.9	20.2	19.1
CGH-118	13.4	15.3	19.5	16.1
CGH-128	17.2	18.2	20.4	18.6
CGH-104	13.5	15.7	18.6	15.9
CGH-69(check)	12.5	14.5	16.5	14.5
Mean	15.3	17.6	20.0	
	(A)	(B)	(AXB)	
SE(m)	0.08	0.04	0.14	
CD	0.23	0.12	0.41	

3.1.3 Germination percentage

Among the genotypes, the germination showed variation at the 10th day of observation. The genotype CGH-72 recorded highest germination (95.6%) followed by the genotype CGH-16 (94.8%) whereas the lowest germination was recorded in the check CGH-69 (78.9%) as shown in Table 3. Genotypes also showed variable response in germination to the application of external nitrogen levels. The germination percentage increased from 80.7% in N0 to 97.0% in N2. The interaction effect of genotype and N levels

on germination was found to be prominent. The genotypes CGH-85, CGH-128 and CGH-118 were recorded 100% of germination due to N2 level of N application. However at N0 Level of N application the genotype CGH-72 recorded the highest germination 93.3% against the check (63.3%). The variation in germination percentage among the genotypes may be due to their differences in genetic makeup and seed nutrient reserves, which might have influenced seed viability and early seedling establishment. The findings are in agreement with Hara and Toriyama (1998) ^[12].

Table 3: Effect of different nitrogen levels on germination percentage (%)

Genotype(A)	N0	N1	N2	Mean
CGH-219	73.3	86.7	96.7	85.6
CGH-278	86.7	90.0	96.7	91.1
CGH-72	93.3	96.7	96.7	95.6
CGH-13	66.7	93.3	96.7	85.6
CGH-41	86.7	93.3	96.7	92.2
CGH-16	91.0	96.7	96.7	94.8
CGH-85	73.3	93.3	100.0	88.9
CGH-118	86.7	96.7	100.0	94.4
CGH-128	90.0	93.3	100.0	94.4
CGH-104	76.7	93.3	96.7	88.9
CGH-69(check)	63.3	83.3	90.0	78.9
Mean	80.7	92.4	97.0	
	(A)	(B)	(AXB)	
SE(m)	1.89	0.99	3.32	
CD	5.37	2.80	9.3	

3.1.4 Seedling vigour

The data presented in Table 4 showed that among the genotypes, seedling vigour varied significantly due to kernel N content. The highest vigour was noted in genotype CGH-278 (55.5g) followed by CGH- 16 (46.9g) and lowest vigour was recorded in CGH-69 (14.2g). Genotypes also showed variable response in seedling vigour with the application of external nitrogen levels. The mean seedling vigour increased from 18.4 g in N1 to 39.1 g in N2 as presented in Table 4. The interaction effect of genotype and N levels on seedling vigour was found to be prominent. The genotype CGH -278 recorded the highest vigour 72.2cm due to the N2 level of N application. This was followed by the genotype CGH-16(60.1cm) at N2 level. However, at N0 level of N application the genotype CGH-278 was recorded highest vigour 37.5 cm against the check (7.6cm). Higher seed nitrogen improved overall seedling establishment and growth, that leads to greater vigour. The evidence was in support of Welch (1977) ^[21], Evans and Bhatt (1977) ^[10], Wen (2018) ^[22].

Table 4: Effect of different nitrogen levels on seedling Vigour (g seedling⁻¹)

Genotype	N0	N1	N2	Mean
CGH-219	11.7	22.2	33.0	22.3
CGH-278	37.5	56.8	72.2	55.5
CGH-72	21.4	28.8	36.8	29.0
CGH-13	8.9	16.8	23.8	16.5
CGH-41	22.3	30.5	45.4	32.7
CGH-16	34.6	46.1	60.1	46.9
CGH-85	11.2	23.4	33.3	22.6
CGH-118	14.9	18.9	35.7	23.2
CGH-128	14.6	17.3	27.0	19.6
CGH-104	17.8	31.8	42.5	30.7
CGH-69(check)	7.6	13.8	21.3	14.2
Mean	18.41	27.87	39.18	
	(A)	(B)	(AXB)	
SE(m)	1.20	0.62	2.12	
CD	3.40	1.77	5.90	

3.1.5 seedling dry weight

The results presented in table 4 indicated that among the genotypes, the variation for dry weight was found to be significant and the highest dry weight was recorded for genotype CGH-278 (0.60 g) followed by CGH-16 (0.49 g) and lowest dry weight was recorded for CGH-69 (0.18 g). Genotypes also showed variable response in dry weight with the application of external nitrogen levels. The dry weight ranges from N0 (0.22g) to N2 (0.41g) as reported in Table 5. The interaction effect of genotype and N levels on seedling dry weight was found to be prominent. the genotype CGH- 278 was recorded highest dry weight of 0.75g due to N2 level of N application. This was followed by the genotype CGH-16 (0.62g) at N2 level. However, at N0 level of N application the genotype CGH- 278 was recorded the highest dry weight 0.43g against the check(0.12g). The increase in dry weight indicates efficient nitrogen utilisation and biomass accumulation. These observations were in accordance with the reports of Lowe *et al.* (1979), Steer and Harrington (1986), Ebrahimian *et al.* (2013)^[7], and Alves de Anicésio *et al.* (2015)^[3].

Table 5: Effect of different nitrogen levels on seedling dry weight (g seedlings⁻¹)

Genotype	N0	N1	N2	Mean
CGH-219	0.16	0.26	0.34	0.25
CGH-278	0.43	0.63	0.75	0.60
CGH-72	0.23	0.30	0.38	0.30
CGH-13	0.13	0.18	0.25	0.19
CGH-41	0.26	0.33	0.47	0.35
CGH-16	0.37	0.48	0.62	0.49
CGH-85	0.15	0.25	0.33	0.25
CGH-118	0.17	0.19	0.36	0.24
CGH-128	0.16	0.19	0.27	0.21
CGH-104	0.23	0.34	0.46	0.34
CGH-69(check)	0.12	0.17	0.25	0.18
Mean	0.22	0.30	0.41	
	(A)	(B)	(AXB)	
SE(m)	0.01	0.006	0.02	
CD	0.03	0.016	0.052	

4. Conclusion

The present study revealed that kernel nitrogen content significantly influenced early seedling growth, dry matter, among the selected safflower genotypes. Genotypes with higher kernel nitrogen exhibited better seedling performance than the check under different external nitrogen levels. The observed variation among the genotypes indicates that kernel nitrogen content as an important parameter that can be used as a criterion for identifying the nitrogen-efficient safflower genotypes. These findings may contribute to future breeding programmes aimed at developing cultivars with improved nitrogen use efficiency.

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