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Morpho-Physiological Adaptations of Drought-Resilient and Susceptible Brassica napus Genotypes to Sulphur-Coated Urea Fertilization

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ABSTRACT
This study focuses on screening drought-tolerant and sensitive genotypes of Brassica napus L. (an important oilseed crop in Pakistan) and investigates their morpho-physiological responses to sulphur-coated urea (SCU) under drought conditions. Genotypes were categorized into highly drought tolerant (HDT), moderately drought tolerant (MDT), and highly drought sensitive (HDS) through hierarchical cluster analysis. Out of 100 genotypes, 20 were HDT, 30 were MDT, and 50 were HDS. Three genotypes from each category were subjected to two drought levels (80% field capacity and 50% field capacity) and were treated with SCU, urea, or no nitrogen. Key parameters such as leaf relative water content, chlorophyll content, excised leaf water loss, and membrane stability index (MSI) were recorded. Drought-tolerant genotypes performed well in both germination and seedling stages, showing higher excised leaf water loss, relative water content, chlorophyll values, seed weight, and yield-related traits. Initially, five concentrations of PEG-6000 (5% to 25%) were tested to determine optimal levels for screening drought tolerance. Results showed that 25% PEG-6000 induced 50% drought injury index (DII), making it the most promising concentration. Various morpho-physiological traits such as germination rate, shoot/root length, fresh and dry weight, and drought tolerance indices were assessed. The study concluded that drought-tolerant genotypes responded positively to SCU application, making them suitable for cultivation in water-scarce regions of Pakistan.

INTRODUCTION
Brassica napus L. is the most important oil seed crop of Pakistan (Sabagh *et al.*,2019). It is principally cultivated in irrigated as well as rainfed areas of Punjab, Sindh, and Khyber Pakhtunkhwa provinces on an area of 0.077 mha with production of 0.049 million tons (Pakistan Bureau of Statistics, 2020-21). The average yield of *B. napus* is 812 kg/ha for below the global average. The local production of edible oil is Estimated at 0.374 million tons against required 3.291 million tons (Pakistan Bureau of Statistics, 2020-21). The indigenous oil output is not keeping pace with the expanding demand of ever-growing population, forcing the country to import 2.917 million tons of

edible oil annually worth Rs. 574.199 billion (US$ 3.419 billion), putting strain on foreign exchange reserves. Indeed, despite an agricultural country, Pakistan ranks third among the largest importers of oilseeds and edible oil (Zia *et al.*,2020).

Drought stress is menace for crop production across the globe and alone can result in 40 % to 60 % global agricultural output losses (Mustafa *et al.*, 2022). The most crucial stage in the life cycle of B. napus that is affected by drought stress is germination and the early seedling stage. In Pothwar region of Punjab, *B. napus* is cultivated as rainfed crop, wherein the crop faces drought stress at germination and early growth stages. Other than germination (Shah *et al.*, 2022), the morpho-physiological and biochemical processes of plants, including chlorophyll content, relative water content, protein synthesis, enzymatic activity (Zhu *et al.*, 2021), stomatal conductance, seed yield, cell division, leaf growth, leaf area, plant height, root and shoot growth, root-shoot ratio, no, of branches, seed per pod and less yield are adversely affected due drought stress (Hussain *et al.*, 2021).

Generally, water stress causes developmental, biochemical, and physiological changes, and the sort of response of *B. napus* relies on numerous parameters like stress intensity (SI), given stress duration and genotype (Choudhary *et al.*, 2021). Plants use a variety of morphological and physiological adaptations to combat drought stress (Priya *et al.*, 2021). For example, chlorophyll content, RWC, ELWL are decreased while proline, glycine betaine and K^+ contents are increased in response to drought stress and are frequently reported for appraisal of level of drought tolerance of *B. napus*. (Dąbrowski *et al.*, 2021). Stress tolerance index (DTI) of morpho-physiological traits is a reliable tool for assessing drought tolerance level of genotypes (Priya *et al.*, 2021).

Sulphur is a 4th vital nutrient for oilseed crops after nitrogen, phosphorus, and potassium (Batool *et al.*, 2022). B. napus is sensitive to S deficiency and can reduce both seed quality and yield by 40% (Ali *et al.*, 2017). Besides sulfur is essential for normal plant growth, development, and protein metabolism, and plays an important role in the defense of plants against drought stress (Batool *et al.*, 2022).

Nitrogen occupies a conspicuous place in plant metabolism and had a key role in crop production system (Al-Mushhin *et al.*, 2021). Protein, of which nitrogen is a necessary component, is linked to all critical plant functions. Consequently, to get more crop production, nitrogen application is indispensable and is generally applied in urea form. Leaching, volatilization and denitrification are the most common forms for removing significant amounts of applied urea from arable farms across the world (Hniličková *et al.*, 2019). Strategies are being formulated to reduce nitrogen losses and to improve NUE from urea by coating the urea with various biodegradable materials and nutrients (Puvanitha *et al.*, 2017). Sulphur coated urea (SCU) can act as a slow-release fertilizer and increases nitrogen use efficiency, improves plant growth, and reduces water pollution, as compared with water soluble fast-release urea (Zhu *et al.*, 2021). Application of sulphur coated urea improves nutrient use efficiency (NUE), diminishing nutrient removal rate with reduced environmental hazards. (Farouk and Arafa, 2018). Controlled release fertilizers (CRFs) have been because they are made available to the targeted plants at the correct rate for a longer period of time, substantial research has been done to produce a safer, more affordable, and more effective method of delivering nutrition (Gil-Ortiz *et al.*, 2022). Application of Controlled release fertilizers can decrease fertilizer application rate by means of 20 to 30% of the recommended one to achieve the same yield (Minato *et al.*, 2020).

The study was helpful to ascertain the effectiveness and reliability of screening criteria at germination and seedling stage, in addition to identify reliable morpho-physiological and yield-related traits to identify drought tolerant and sensitive genotypes/line ones while taking the afore mentioned difficulties into consideration for selection of drought sensitive and tolerant genotypes/germplasm of *B. napus* by achieving the following objectives.

The proposed piece of work will be carried out with objective of:

1. Evaluation of canola seed germplasm/genotypes for drought tolerance at germination and seedling stage.
2. Study effect of sulphur coated urea on drought tolerance of *B. napus.*
3. Nitrogen use efficiency response of *B. napus* to coated and uncoated urea in control and stress conditions.

MATERIALS AND METHODS

Study pertaining to morpho-physiological responses of drought tolerant and sensitive canola seed (*Brassica napus* L.) genotypes to Sulphur coated urea was conducted at Department of Agronomy, PMAS Arid Agriculture University Rawalpindi.

Collection of Germplasm

Various genotypes and inbred lines/accessions of *Brassica napus* were collected from NARC and Ayub Agricultural Research Institute Faisalabad and other sources. In addition to the given below germplasm, effort was made to collect maximum number of germplasms of *B. napus*.

Pakola, Hyola-40, PARC Canola hybrid, Dunkled, Faisal canola, RBN-03046, RBN-11049, AARI Canola, Con-II(Canola), Shiralee, Hope-09, Durr-e-NIFA, Abasian-95, Abasian-95, RBN-04722, Punjab Canola, NIFA Gold, Punjab Sarson, Bulbul, Canola Raya (juncia), 19-h(Rapa), Super canola.

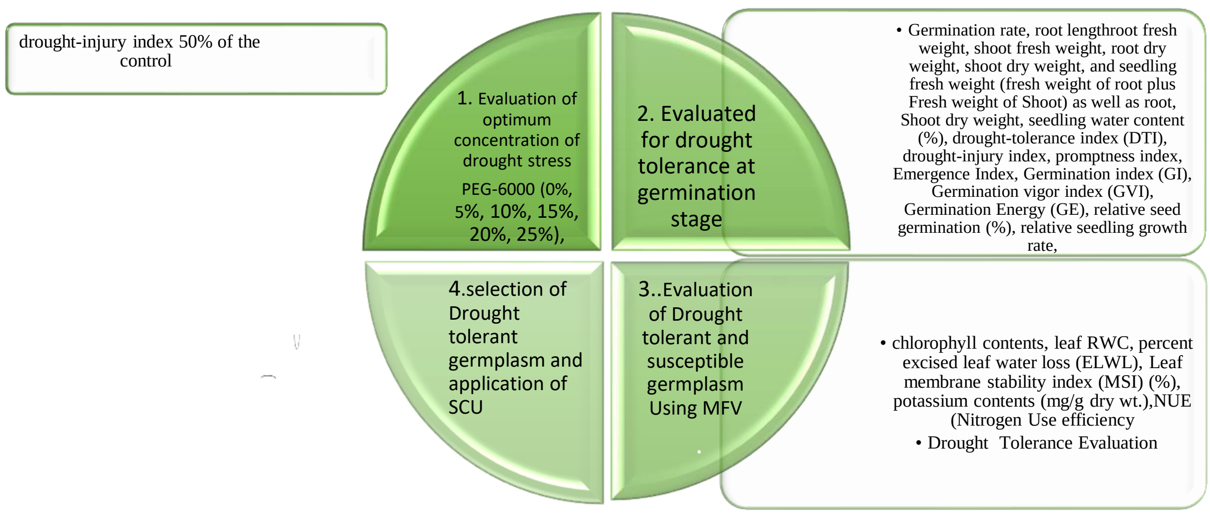
Evaluation of Germplasm for drought Tolerance at Germination and Seedling Stage

At first step of the experiment, the sub-lethal (optimum) concentration of PEG-6000 stress within 5%, 10%, 15%, 20%, 25%, along with control were determined using method of Wu *et al.*, (2019) with little modification. Five seeds of each germplasm/genotype were randomly taken, and a composite sample was made. The composite sample was surface sterilized with 70% alcohol for 15 min followed by washing with five changes of sterilized distal water. The sample was divided into six equal parts with an equal number of seeds. Each sample was germinated in Plastic boxes (20cm ×13cm ×7cm) with tight lid. The boxes were equipped with two layers of blotter paper covering the deck completely. The blotter papers were submerged in different solutions of PEG-6000 (0, 5%, 10%, 15%, 20%, 25%, 30% w/v solution) and was immediately removed, holding vertically for two minutes to drain excessive water. Blotter papers treated with Distilled water were taken as control. The blotter papers were kept saturated till end of the study by applying equal volume of Distilled water on daily basis. The germination and seedling growth indices (germination %, germination rate, root length, shoot length, seedling fresh and dry weight, shoot elongation rate, root elongation rate) were recorded for 10-15 days. The seeds were considered germinated with radicle length of ≥2 mm. The stress level which inhibits 50-60% of studied traits was taken as sub-optimum stress level for further studies.

In the second step, after evaluating optimum concentration of PEG-6000, the germplasm/genotypes were evaluated for drought tolerance at germination stage and early seedling stage based on fuzzy membership functional value (Wu *et al.*, 2019). Fifteen seeds of each genotype were randomly selected, surface sterilized and was kept for germination with optimum concentration of PEG-6000 along with control (distill water) in roll blotter papers. In each piece of blotter paper, the seeds were divided into three groups each with 5 seeds. Each group of seeds were designated as a separate replication. The saturated rolled blotter papers were kept in jars with a tight lid. Various morpho-physiological traits (germination rate, root and shoot length, root, shoot length ratio, root and shoot fresh weight, seedling total fresh weight and dry weight, germination index, germination energy, germination vigor index, drought injury index and seedling water content) were recorded. The MFV value of all traits were computed (Wu *et al.,* 2019). The average of MFVs of all traits were determined for cluster analysis and grouping the genotypes into drought tolerant and sensitive ones. The principal component analysis was performed to group and identify drought tolerant and sensitive genotypes.

In the 3rd step of study, drought susceptible and tolerant genotypes were selected based on performance at germination and early seedling stage. The selected germplasm was grown in pots under two treatments of fertilizers (i) the sulphur coated urea and (ii) Urea sole imposed one week after emergence of seed. Various morpho-physiological parameters (leaf relative water content, excised leaf water loss, proline content, chlorophyll spade value, NUE, MSI, drought injury index) were recorded 25-35 days after sowing, depending upon growth of plants.

Figure 1
Flow chart of materials and methods



Data Collection
The data pertaining to following parameters/traits were collected:

Germination Rate
Germination of seed was recorded daily for seven days. The seed was considered germinated if radicle length is greater than 2mm. The number of germinated seeds on day 7 was recorded. The germination % was find out by using following formula:

$$Germination\ rate\ (\%) = \frac{No.of\ seed\ germinated}{Total\ No.of\ seeds\ cultured\ for\ germination} \times 100$$

Root and Shoot Parameters
The seedlings with abnormal germination (faster or slower germination) were removed and uniform seedlings were selected and maintained. The data pertaining to root length, shoot length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight and seedling fresh weight (fresh weight of root + fresh weight of shoot) and root, shoot dry weight was recorded 10-12 days after sowing (Wu *et al.*, 2019). For dry weight the samples were dried in oven at 80 °C till constant weight.

Drought-tolerance index (DTI)
DTI is the ratio of a trait (root length, shoot length etc.) of germplasm under drought-stressed condition to that of control (distill water). Each trait of each germplasm has its own DTI (Li *et al.*, 2020).

$$DTI_i = \frac{value\ of\ trait\ in\ the\ \ drought\ stressed\ treatment}{value\ of\ trait\ i\ in\ control\ treatment}$$

Promptness index (PI)
It is a percentage of seeds which germinate at 2nd, 4th, 6th, and 8th day of study and denoted by nd_2, nd_4. nd_6, and nd_8. Promptness index was calculated using following formula:

$PI = nd_2\,(1.0) + nd_4\,(0.8) + nd_6\,(0.6) + nd_8\,(0.4)$

So, nd_2, nd_4, nd_6 and nd_8 had number of seed germinated on 2, 4, 6 and 8 days

Germination index (GI)
The germination index was calculated by the following formula (Li et al.,2020):

$$GI = \sum \frac{\text{Total no. of germinated seeds on } T^{th} \text{day}}{\text{number of days after sowing on which germination was recorded}}$$

Germination Vigor Index (GVI)
Germination vigor index was calculated using formula given by Li et al., (2020) as under:

$$GVI = \sum \frac{\text{Total no. of germinated seeds on } T^{th} \text{day}}{\text{number of days after sowing on which germination was recorded}} \times \text{Average fresh weight of seedling}$$

Leaf membrane stability index (MSI) (%)

MSI was determined following method of Premachandra et al. (1990). In two different sets of test tubes 10.0 ml of double Distilled water were taken. The small pieces of leaf sample (200 mg) were added to the test tubes. One set of the test tubes was warmed for 30 min at 40 ºC in a water bath. The electrical conductivity (EC) of heated samples were recorded (C1). The second set of test tubes were incubated in boiling water in the water bath at 100 ºC for 15 minutes and their EC was noted as above (C2). MSI was determined by using following formula:

$$\mathrm{MSI} = \left(1 - \frac{\mathrm{C1}}{\mathrm{C2}}\right) \times 100$$

Drought Tolerance Evaluation

The drought tolerance of B. napus was evaluated using the membership function value (MFV) employing the fuzzy comprehensive evaluation method (Chen et al., 2012; Wu et al., 2019). The MFV of drought tolerance was calculated using the following equation (Ding et al.,2018; Wu et al.,2019):

$$\mathrm{Xi} = \frac{\mathrm{X-Xmin}}{\mathrm{Xmax-Xmin}} \times 100\ \%$$

Where,

X_i = MFV of DTI in a specific germplasm/genotype

X = Actual measured value of DTI of a specific genotype/germplasm

X_{max} = maximum values of DTI observed in all inbred lines

X_{min} = minimum values observed of DTI in all inbred lines,

The salt tolerance of the inbred line was assessed using the average value of the MFVs for each characteristic. All inbred lines' MFVs fell between 0 and 1. The mean MFV for each genotype is the average of the MFVs for the following Characteristics: germination rate, root weight, shoot weight, root length, shoot length, and total fresh weight of seedling. Therefore, each genotype had its own mean MFV; the higher the mean MFV, the higher the respective germplasm/drought genotype's tolerance.

Data Analysis

The statistical analysis was performed by the analysis of variance using IBM Statistical package SPSS. The treatment means was compared by Least Significant Difference test (α=0.05). The correlations between the quantitative variables were determined using Pearson correlation coefficient formula.

RESULTS

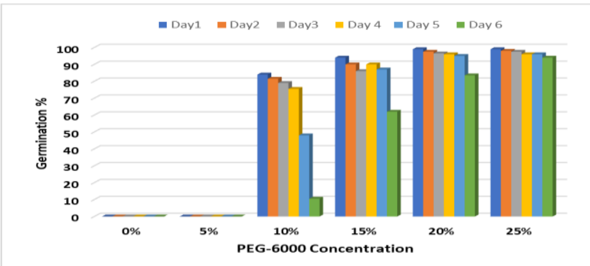
The study comprised of following three experiments depending on the results of preceding ones:

- Determination of optimum concentration of PEG-6000 stress for screening of drought tolerant and sensitive germplasm of *B.napus*
- Evaluation of *B. napus* germplasm for drought tolerance at germination stage
- Study performance of some selected drought tolerant and sensitive germplasm under filed capacity and severe drought stress in the pots.

Determination of Optimal Drought Stress Concentration

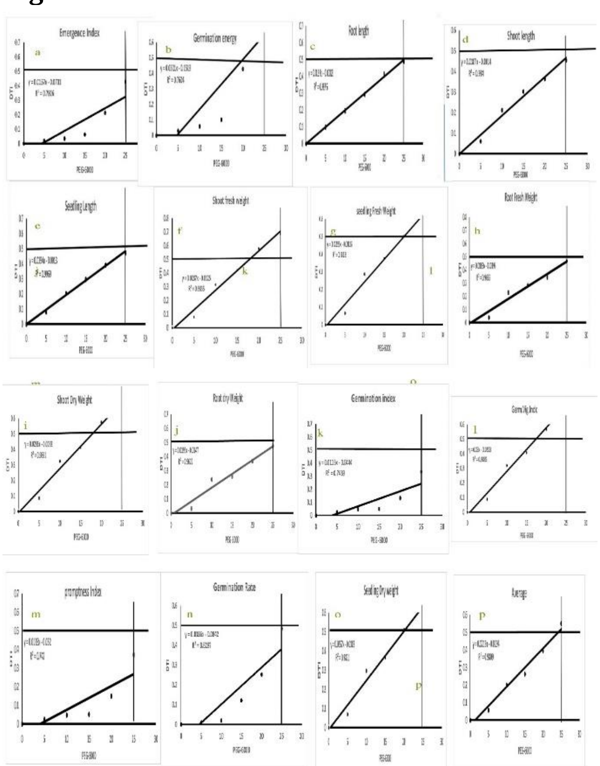
At the first step, the optimum concentration of PEG-6000 within 5%, 10%, 15%, 20%, and 25% percent was given PEG-6000 was evaluated for screening of B. napus germplasm based on various germination indices (Germination rate, germination index, germination energy, germination vigor index, germination speed, promptness index, shoot length, root length, seedling length, shoot fresh weight, root fresh weight, seedling fresh weight, root dry weight, shoot dry weight and seedling dry weight). A representative sample of 100 genotypes of B. napus was prepared giving representation to each genotype. The representative sample comprised of 5 seeds from each genotype. Seeds with radical length of ≥2 mm were considered as a germinated seed. Drought stress level at which drought injury index to studied trait was 50% of control was considered the optimum stress level of PEG-6000 (Wu et al., 2019) for the trait under study. The drought injury index (DII) of all the studied parameters was computed by mathematical model. According to this, 50% DII for germination rate was recorded against 25% PEG-6000 solution promptness index (43.1 %), germination index (47 %), emergence index (36.31 %) (177mM), germination vigor index (17.3 %), germination energy (20.3 %), shoot length (26.8 %), root length (25.1 %), seedling length (25.8%), shoot fresh weight (17.85%), root fresh weight (26.9 %), seedling fresh weight (20 %), shoot dry weight (17.8%), root dry weight (26.3 %) and seedling dry weight (19.8 %) (Fig. 4.1). The mean of all DTI of all the parameters was also subjected to a mathematical equation and 25 % PEG-6000 was found promising with 50% DII (Fig. 2).

Figure 2
Germination of B. napus under various PEG-6000 concentrations.



Germination and early seedling stages are the most sensitive to environmental conditions and are considered ideal for drought tolerance evaluation. All oilseed crops including B. napus have small seeds and also less tolerance to drought at germination stage

Figure 3



Mathematical equations showing the effect of various concentrations of PEG-6000 on (a) Germination Rate (b) Promptness Index (c) Germination Index (d) Germination Speed (e) Germination Vigour Index (f) Germination Energy (g) Shoot Length (h) Root Length (i) Seedling Length (j) Shoot Fresh Weight (k) Root Fresh Weight (l) Shoot Dry Weight (m) Root Dry Weight (n) Seedling Fresh Weight (o) Seedling Dry Weight (p) Germination and Seedling indices Average.

Screening of B. Napus Germplasm at Germination Stage

Seeds of 100 genotypes of brassica were germinated at optimum stress level of PEG-6000 based on the previous part of the study. The drought tolerance index at 25 % PEG-6000 for germination rate (GR), germination index (GI), germination energy (GE), germination vigor index (GVI), germination speed (GS), promptness index (PI), shoot length (SL), root length (RL), seedling length (SDL), shoot fresh weight (SFW), root fresh weight (RFW), seedling fresh weight (SDFW), root dry weight (RDW), shoot dry weight (SDW) and seedling dry weight (SDDW) was computed. High DTI value indicated the smaller effect of induced salinity stress on the studied traits and vice versa. Among the germination traits, germination energy (GE) of all the genotypes was more affected by PEG-6000 stress (25%).

Frequency Distribution of B. Napus Genotypes Based On germination and Seedling Indices in Response to Salt Stress

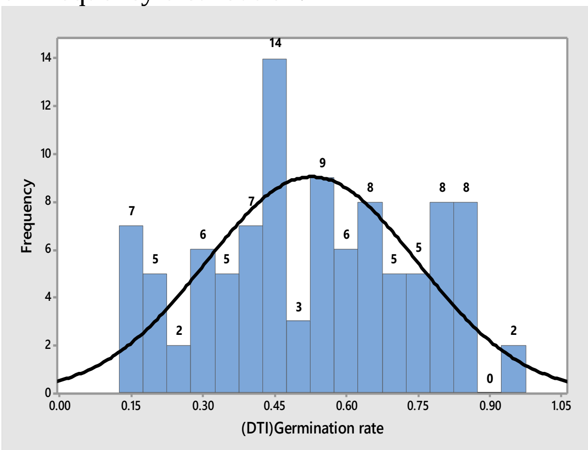
The frequency distribution of B. napus genotypes based on drought tolerance index of germination and seedling traits showed that root fresh weight was less effect by PEG-6000 induced stress, whereas germination energy (GE) was highly prone to drought stress.

Germination Traits

Germination rate

Frequency distribution for germination rate (GR) showed that 20 genotypes had DTI values ranging from 0.15 -0.30, while 26 genotypes had DTI values from 0.31-0.45, 18 genotypes had DTI values ranging from 0.46-0.60, 18 genotypes had DTI value from 0.61-0.75 and 18 genotypes had DTI value from 0.76-1.05 (Fig. 4).

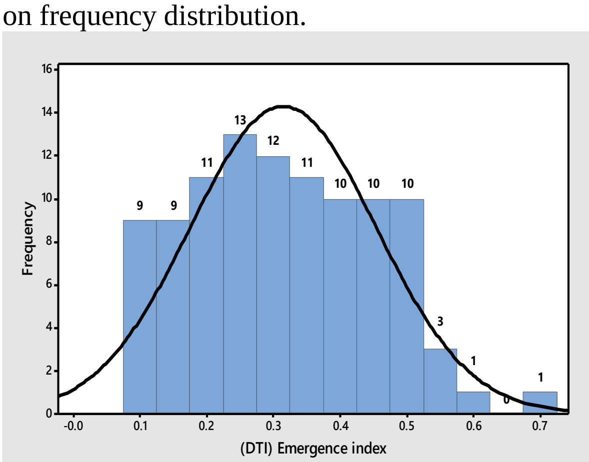
Figure 4
Germination Rate (GR) of germination data based on frequency distribution.



Germination index

For germination index (GI), 9 genotypes had a minimum DTI value from 0.0-0.1, 20 genotypes had DTI value from 0.11-0.2, 25 genotypes had DTI value from 0.21-0.3, 21 genotypes had DTI value from 0.31-0.4, 20 genotypes had DTI value from 0.41-0.5, genotypes had DTI value from 0.51-0.6 and only 1 genotype (Raya) exhibited maximum DTI value from 0.61-0.7 (Fig. 5).

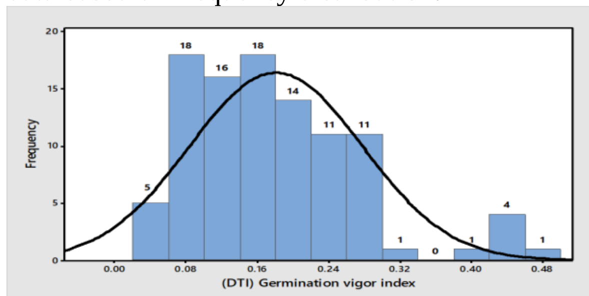
Figure 5
Germination Index (GI) of germination data based on frequency distribution.



Germination vigor index

For germination vigor index (GVI), 23 genotypes showed the maximum DTI value from 0.0-0.08, whereas 34 genotypes had DTI values from 0.09-0.16, 35 genotypes had values from 0.17-0.24, 12 genotypes had DTI between 0.25-0.32 and 6 genotypes had DTI value from 0.33-0.48 (Fig. 6).

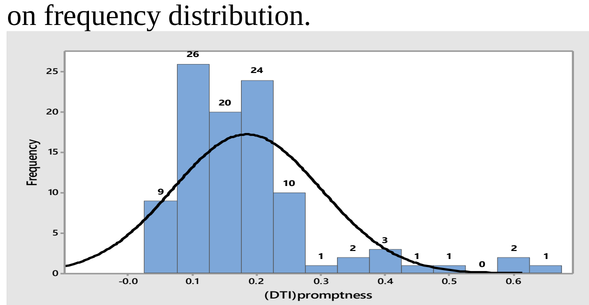
Figure 6
Germination Vigor Index (GVI) of germination data based on frequency distribution.



Promptness index

The DTI of promptness index (PI) showed that 35 genotypes had DTI values from 0.0-0.1, 44 genotypes had DTI values from 0.11-0.2, 11 genotypes had DTI values from 0.21-0.3, 5 genotypes had DTI value from 0.33-0.4, 5 genotypes had DTI value from 0.41-0.6, (Fig. 7).

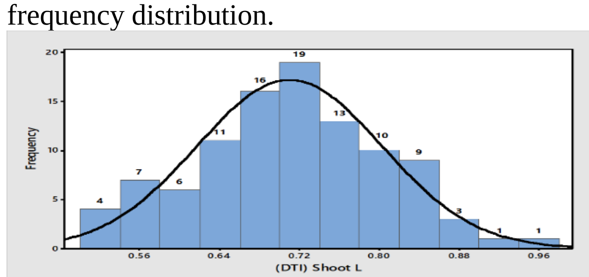
Figure 7
Promptness Index (PI) of germination data based on frequency distribution.



Shoot length (cm)

DTI of shoot length (SL) indicated that 11 genotypes had DTI values from 0.00-0.56, 17 genotypes had DTI values from 0.57-0.64, 35 genotypes had DTI values from 0.65-0.72, 23 genotypes had DTI value from 0.73-0.80, 12 genotypes had DTI values from 0.81-0.88, 2 genotypes had DTI value from 0.89-0. (Fig. 8).

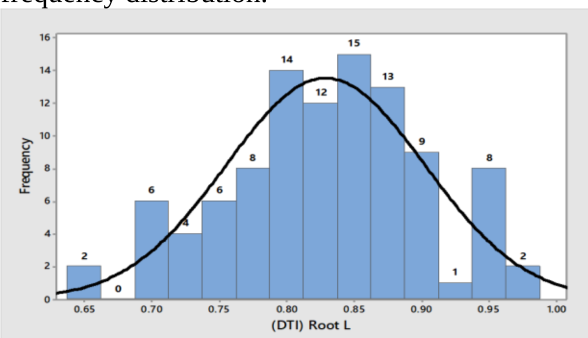
Figure 8
Shoot Length (SL) of germination data based on frequency distribution.



Root length (cm)

The DTI of root length (RL) showed that 8 genotypes had DTI values from 0.65-0.70, 10 genotypes had DTI values from 0.71-0.75, 22 genotypes had DTI values from 0.76-0.80, 27 genotypes had DTI value from 0.81-0.85, 22 genotypes had DTI value from 0.86-0.90, 9 genotypes had the DTI value ranging from 0.91-0.95 and two genotypes (Dunkle and CBN-39) had DTI value from 0.96-1.00 (Fig. 9).

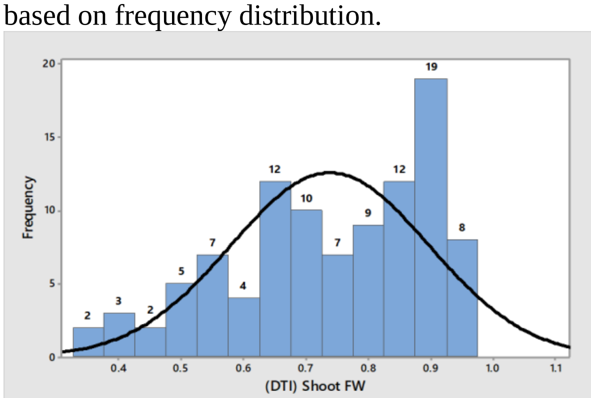
Figure 9
Root Length (RL) of germination data based on frequency distribution.



Shoot fresh weight (g)

For shoot fresh weight (SFW) five genotype had DTI value from 0.0-0.4, 7 genotypes had DTI value from 0.41-0.5, 11 genotypes had DTI value from 0.51-0.6, 22 genotypes had DTI value from 0.61-0.7, 16 genotypes had DTI value from 0.71-0.8, 31 genotypes had DTI value from 0.81-0.9, 8 genotypes had DTI value from 0.91-1. (Fig. 10).

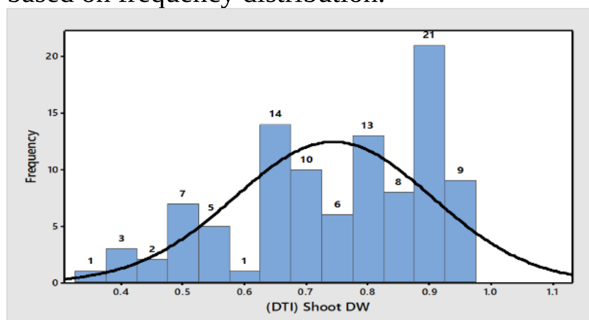
Figure 10
Shoot Fresh Weight (SFW) of germination data based on frequency distribution.



Shoot dry weight (g)

For shoot dry weight (SDW) four genotypes had DTI values from 0.0-0.4, 9 genotypes had DTI values from 0.41-0.5, 6 genotypes had DTI value from 0.51-0.6, 24 genotypes had DTI values from 0.61-0.7, 19 genotypes had DTI values from 0.71-0.8, 29 genotypes had DTI values from 0.81-0.9 and 9 genotypes had DTI values from 0.9-1.1 (Fig. 11).

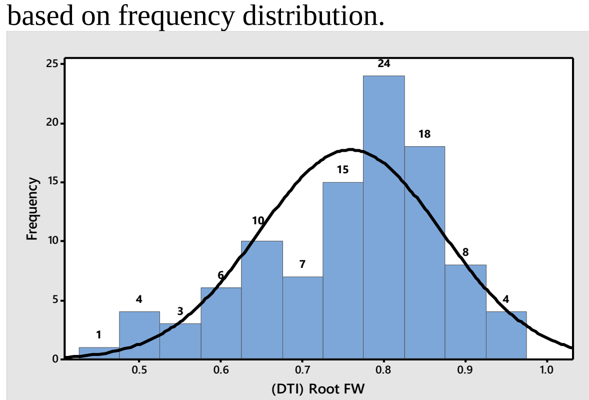
Figure 11
Shoot Dry Weight (SDW) of germination data based on frequency distribution.



Root fresh weight (g)

For root fresh weight (RFW) five genotypes had DTI values from 0.0-0.5, 9 genotypes had DTI values from 0.51-0.6, 17 genotypes had DTI values from 0.61-0.7, 39 genotypes had DTI values from 0.71-0.8, 26 genotypes had DTI values from 0.81-0.9 and four genotypes (CBN-11, CBN-74, CBN-42 and CBN-63) had DTI values from 0.91-1.0 (Fig. 12).

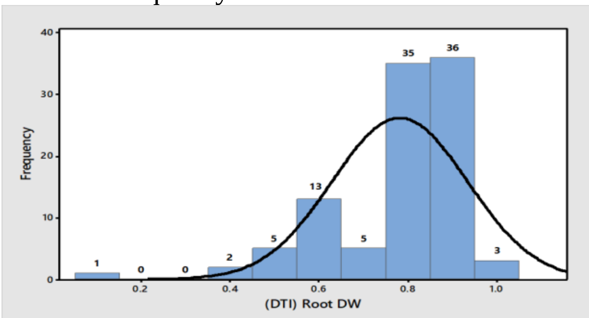
Figure 12
Root Fresh Weight (RFW) of germination data based on frequency distribution.



Root dry weight (g)

For root dry weight (RDW) three genotypes (CBN-3, CBN-9 and CBN-79) had DTI values from 0.0-0.4, 18 genotypes had DTI values from 0.41-0.6, 40 genotypes had DTI values from 0.61-0.8, 39 genotypes had DTI values from 0.81-1.0 (Fig. 13).

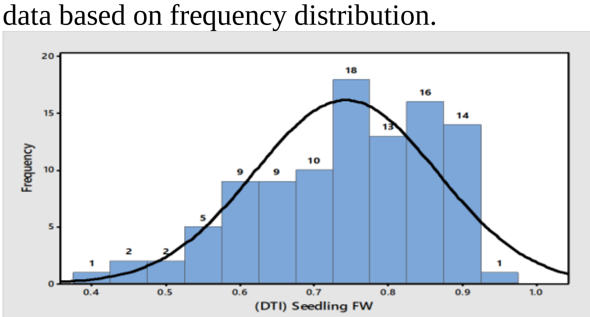
Figure 13
Root Dry Weight (RDW) of germination data based on frequency distribution.



Seedling fresh weight (g)

For seedling fresh weight (SDFW) only five genotypes had DTI value from 0.4-0.5, 14 genotypes had DTI values from 0.51-0.6, 19 genotypes had DTI values from 0.61-0.7, 31 genotypes had DTI values from 0.71-0.8, 30 genotypes had DTI values from 0.81-0.9 and one genotypes had DTI values from 0.91-1.0 (Fig. 14).

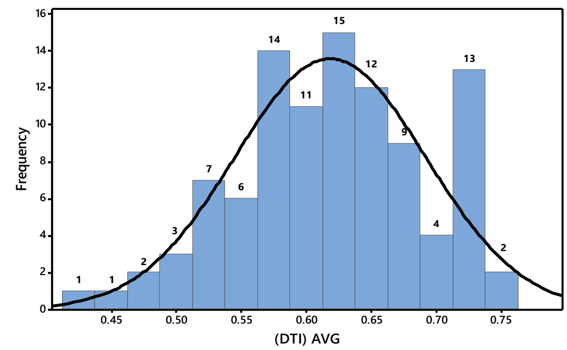
Figure 14
Seedling Fresh Weight (SDFW) of germination data based on frequency distribution.



Mean DTI of Germination and Seedling Indices

The average DTI of germination parameters (germination rate (GR), germination index (GI), germination energy (GE), germination vigor index (GVI), germination speed (GS), promptness index (PI) and seedling parameters (shoot length (SL), root length (RL), seedling length (SDL), shoot fresh weight (SFW), root fresh weight (RFW), seedling fresh weight (SDFW), root dry weight (RDW), shoot dry weight (SDW) and seedling dry weight (SDDW) was computed. two genotypes exhibited DTI values from 0.0-0.45, five genotypes had DTI values from 0.46-0.50, 13 genotypes had DTI values from 0.51-0.55, 25 genotypes had DTI values from 0.56-0.60, 27 genotypes had DTI value from 0.61-0.65, 13 genotypes had the DTI value form 0.66-0.70 and 15 genotypes exhibited DTI value from 0.71-0.75. (Fig. 15).

Figure 15
Average of all germination data based on frequency distribution.



Evaluation of B. *Napus* Genotypes on The Basis of Membership Functional Value (MFV) and Cluster Analysis

The Membership Function Value (MFV) is a screening method for different stress (salinity and drought) conditions employing fuzzy comprehensive evaluation method. The MFV was computed as proposed by Wu et al. (2019).

$$Xi = \frac{X - Xmin}{Xmax - Xmin} \times 100\ \%$$

Where:
X_i = MFV of DTI of a specific germplasm/genotype
X = Actual measured value of DTI of a specific genotype/germplasm
X_{max} = Maximum values of DTI observed within all inbred lines
X_{min} = Minimum values of DTI observed within all inbred lines

The mean MFV of all traits was subjected to Hierarchical Cluster Analysis for grouping of genotypes into four groups viz: highly drought tolerant (HDT), moderately drought tolerant (MDT) and highly drought sensitive (HDS). Based on Hierarchical Cluster Analysis and mean MFV of all germination indies, the genotypes were divided into three groups; (1) 20 genotypes (Faisal canola, RBN-03046, RBN-11049, NARC Sarson, CBN-6, CBN-11, CBN-12, CBN-25, CBN-39, CBN-45, CBN-47, CBN-48, CBN-49, CBN-52, CBN-69 and CBN-73) were highly drought tolerant (HDT) with mean MFV value ranged from 0.596-0.760; (2) 30 genotypes were moderate drought tolerant

(MDT) with mean MFV value ranged from 0.475-0.574; (3) 50 genotypes were highly drought sensitive (HDS) with mean MFV value ranged from 0.196-0.470 (Fig. 3.17). The mean MFV indicated that majority of the genotypes were all in groups of highly drought sensitive and moderate drought sensitive.

Figure 16

Frequency distribution of mean MFV of germination indices of B. *napus* genotypes. HDT (Highly Drought Tolerance), MDT (Moderate Drought Tolerance), and HDS (Highly Drought Sensitive).

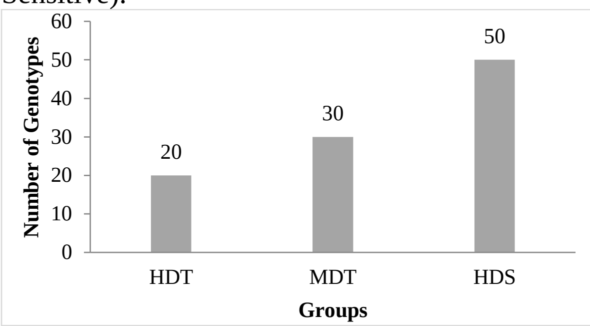
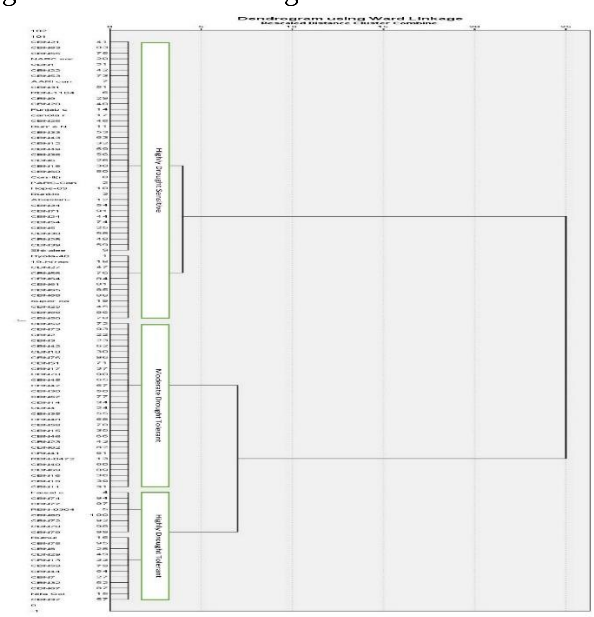


Figure 17

Hierarchical Cluster Analysis of mean MFV of germination and seedling indices.



Morpho-Physiological Responses of Some Selected Drought Tolerant and Sensitive Genotypes of B. *Napus* at Whole Plant Level

After the selection of some drought tolerant , moderately drought tolerant and drought sensitive plant they were subjected to grow in pots in a control environment (Glass House) to check the effect of drought on two field capacity levels one with 80% fc maintained as control while other at 50% Fc maintained at severe drought conditions three varieties were selected after the cluster analysis of seedling and germination indices the varieties are Faisal canola (HDT),NRAC-sarsoon (MDT) and Super canola (HDS) they were given different doses of fertilizer like SCU and Sole urea along with control. Quality parameters including the Leaf area, Chlorophyll content, membrane stability index, relative water content, and potassium content were measured as discussed below.

Leaf Relative water content (%)

The statistical analysis shows that the means of all the three varieties were significantly different from each other. The leaf relative water content of Faisal canola having the highest LSD mean of 88.50 followed by Narc-Sarsoon and Super canola having the LSD mean 83.80 and 80.80 respectively. The 80% FC (control) field capacity has the highest relative content mean of 88.30 followed by 50% FC (severe drought) having the leaf relative content mean LSD of 80. 40. The relative water content of Sulphur coated Urea has the highest mean LSD of 86.83 while the control has the lower leaf relative water content mean LSD of 81.78 after Sole Urea having the mean LSD of 84.44. Sulphur coated Urea Treatment performed best to retain high moisture content. The relation between variety and stress was significant in the stress the variety Faisal canola drought tolerant variety performed best having the mean of 94.00 while the drought sensitive variety NARC-sarsoon performed very low having the mean of 78.88. Moreover, in the relation between the Variety and fertilizer the variety Faisal canola performed best with sulphur coated urea having the mean of 91.66 as compared to sole urea having the mean of 89.66 and control taking the mean of 84.16. Similarly in relation between stress and fertilizer the LRWC of sulphur coated urea having the highest mean LSD of 90.55 in 80% FC as well as in 50 % Fc taking LSD mean of 83.11 respectively. Thus, the control release fertilizer SCU performed best in both FC as well as in variety treatments as shown in below table. Leaf relative water content in leaves directly reflects plant response to drought stress. As the extent of drought stress increased, LRWC showed a gradual decrease in drought-tolerant and drought-sensitive genotypes.

Table 1
Effect of sulfur coated and uncoated urea on LRWC of drought tolerant and sensitive genotypes of B. napus under various moisture.

	80% FC			50%FC			
	V1	V2	V3	V1	V2	V3	F mean
F1	88.67 c	86 de	84 ef	79.67 h	77 ij	75.33 j	81.78 c
F2	97.33 a	91.67 b	82.67 fg	86 de	81.67 fgh	81.67 fgh	86.83 a
F3	96 a	87 cd	81.33 gh	83.33 fg	79.33 hi	79.67 h	84.44 b
Mean		88.30 a			80.40 b		
Varieties	V1			V2		V3	
Mean	88.50 a			83.80 b		80.80 c	

LSD values: moisture x variety x Fert =2.3803; variety=0.9717; Fert=0.9717; moisture=0.7934 Values sharing common letters did not differ significantly (α=0.05)

Membrane stability index (%)
The statistical analysis showed that high membrane stability index was recorded in 50% Fc where sulphur coated urea was applied as compared to sole urea. Therefore, to provide the nutrient for long duration under water shortage condition the sulphur coated urea results best by reducing the ammonia losses as compared to sole urea.

Table 2
Effect of moisture regimes on MSI of B.napus genotypes.

	F1	F2	F3	Mean
80% FC	0.44 d	0.44 d	0.61 c	0.49 B
50% FC	0.64 c	0.69 a	0.65 b	0.66 A
Mean	0.54 B	0.56 C	0.63 A	

LSD value of stress x fertilizer=0.0412, Means were separate using least significant difference test at 5% level of probability.

The interaction between the variety and fertilizer was found significant, application of SCU increase the membrane stability index of drought susceptible variety floored by three applications of sole urea on same susceptible variety to increase the membrane stability index of drought susceptible variety application of sulphur coated urea it can be increased as compared sole urea**.**

Table 3
Effect of sulfur coated and uncoated urea on potassium content under various moisture regimes.

	V1	V2	V3	Mean
F1	0.42 e	0.43 e	0.47 e	0.44 C
F2	0.62 cd	0.64 bc	0.74 a	0.66 A
F3	0.58 d	0.64 bc	0.69 b	0.63 B
Mean	0.54 C	0.57 B	0.63 A	

LSD value of variety x fertilizer=0.0505, Means were separate using least significant difference test at 5% level of probability

Results showed that that drought tolerant variety Faisal canola had the highest membrane stability index in both moisture regimes as compared to moderate drought tolerant variety NARC sarsoon. where the drought susceptible variety super canola the lowest membrane stability index.

Table 4
Effect of moisture regimes on MSI of B.*napus* genotypes.

	V1	V2	V3	Mean
80%	0.60 b	0.56 bc	0.52 d	0.56 B
50%	0.66 a	0.57 bc	0.56 cd	`0.59 A
Mean	0.54 B	0.56 B	0.63 A	

LSD value of stress x variety =0.0412, Means were separate using least significant difference test at 5% level of probability.

DISCUSSION

Slow-release fertilizers, since they are made available to the target at the optimum rate and sustain nutrients in the soil for a longer amount of time, slow-release fertilizers have undergone substantial research to create a safer, more cost-effective, and efficient method of administering nutrients applying to target side at desired rate and nutrient available for a longer period in soil (Gil-Ortiz *et al.*, 2022). In comparison to water soluble fast-release urea, sulphur coated urea fertilizer, such as polymer coated urea pills of urea, acts as a slow-release fertilizer and enhances plant growth, boosts nitrogen use efficiency, and decreases water pollution. (Zhu *et al.*, 2021). Sulphur-coated urea application increases nutrient use efficiency through less frequent dosing and lowers environmental risks by slowing down the rate at which nutrients are removed from the soil by rain or irrigation. (Fertahi *et al.*, 2021). Alternatively, slow-release fertilizer can be used to produce the same yield by reducing fertilizer application rates by 20 to 30 percent of the recommended levels (Minato *et al.*, 2020). Nitrogen and sulphur are fundamental macronutrients to produce good crops. Nitrogen is required consistently in many amounts for production of crop while common urea has more losses due to volatilization and leeching (Asghar et al., 2022). While application of sulphur increases oil contents and Therefore, A promising approach would be to use polymer-coated urea along with sulphur fertilization. (Parveen *et al.*, 2021).

Hamdallah *et al*. (2020) compared the sulphur-coated urea (SCU) with sole urea and found that effectiveness of single application of SCU over split application of soluble N sources. When SCU fertilizers were applied alone or in combination with other sources, dry matter yields significantly increased. In comparison to SCU-30 and SCU-40, SCU-22 consistently increased dry matter yield and N-uptake. Tian *et al.* (2017) found that Slow-release fertilizer gave higher yields than quick release fertilizer by 14.51 percent in both seasons. Maximum seed yields were achieved by SRF4 and QRF3 in each group (2066.97 and 1844.50 kg/hm2, respectively), with SRF3 (1929.97 kg/hm2) and QRF4 (1839.40 kg/hm2) following closely behind. demonstrated an improvement in seed yield of 12.37 percent and a decrease in unit fertilizer rate of 11.01 percent when compared to QRF4 (Mustafa et al., 2022). Under the fertilization of slow-release fertilizers (SRFs), the branch number, pod number, and dry matter weight greatly increased in comparison to SF (Zhang et al., 2020) and the primary factor affecting seed output was the quantity of pods per plant (Al-Taher & Al-Bourky, 2020). By employing total N as the primary fertilizer, delivering enough N during the plant's later growth phases (Ghumman et al., 2022) and, finally, by lowering the soil's residual N content and Boosting N accumulation and N consumption efficiency, SRF considerably aided canola seed growth (Beig et al., 2022).

High level of PEG-6000 significantly decreases the germination rate, germination index and germination vigour index of *Brassica napus*. Only tolerant genotypes give good results under drought stress conditions. Drought can severely affect seed germination, plant growth, flowering, seed yield and quality as well. The degree of these impacts depends on the plant physiological, biochemical and molecular biological processes, as well as the ability of the plant to adapt to drought stress (Massonnet *et al.*, 2016). Drought tolerance in *B.napus* can be determined on the basis of germination and seedling parameters, for example, germination rate, promptness index, germination vigor index and germination index. However, root length and shoot length are very effective parameters to evaluate the level of drought tolerance at seedling stage (Arif *et al*., 2019). Relatively greater root length under stress conditions indicates higher tolerance and vice versa. Present findings agree with those of (Long *et al*., 2013; Chelli-Chaabouni *et al*., 2010). as reported by Ali *et al.*, (2017). When moderate drought conditions further developed into severe drought conditions at the growing season, decreases were found in oleic acid (by3.8%) and saturated FA (by 0.4%), while increases occurred in linoleic acid (by 2.0%) and linolenic acid (by 1.7%). Furthermore, seed oil decreased by 3.2% and protein in meal increased by 3.9% under drought stress). Podder *et al.*, (2020) investigated that genotype with less than 50% germination rate under stress showed decreased shoot length and dry weight accumulation.

Shivay *et al.* (2016) conducted a study to assess sulphur-coated urea (SCU) as a source of sulphur (S) and a nitrogen fertilizer with improved efficiency. Prilled urea (PU) with a 4 to 5% coating Sulphur enhanced wheat grain production by 9.58 to 11.21 percent, nitrogen absorption by 19.06 to 23.94 percent, and sulphur uptake by 21.76 to 29.29 percent as compared to prilled urea alone (Essa et al., 2021). Sulphur levels of 5% SCU met 50% of the wheat crop's sulphur requirements and increased nitrogen recovery efficiency by 60%. SCU provides environmental protection by increasing nitrogen recovery and crop yield (Shah et al., 2022).

Fang *et al*. (2017) conducted an experiment in a split-plot design, with the N fertilizer acting as the main plot and S rates acting as the subplots. The study used PCU and common urea fertilizer (Urea) in combination with two N fertilizer types and three

S rates (0, 60, and 120 kg ha^{-1}). Different finding showed that PCU's N release Characteristics in field conditions closely matched the N needs of cotton, and that using PCU instead of urea significantly increased soil nitrate nitrogen and ammonium nitrogen contents from the first bloom stage to the initial boll-opening stage (Shah et al., 2022) and accessible Sulphur content was increased in full boll setting stage PCU had boll yields that were 7.03–8.91 percent and 5.54–11.17 percent higher than those produced by urea treatments, respectively (Altaf et al, 2021). Sulphur fertilization also enhanced lint yields by 3.77-9.26%, demonstrating a clear relationship between N and S (Ghafoor et al., 2021). The PCU and S fertilizer also improved the N apparent recovery use efficiency (RUE) and agronomic use efficiency (AUE) (Liu et al., 2022). Cotton grown with sulphur fertilizer has the potential to improve fiber quality (Swify et al., 2023) and the physiological characteristics of leaves in addition to yield and nitrogen use efficiency (Gupta et al., 2023).

Parveen *et al.* (2021) performed an experiment on coated urea (PCU) and conventional urea (CU) while S0 (zero), S1 (30), and S2 (60) kg per ha) were the three sulphur fertilizer rates (Shoukat et al., 2023). The application of nitrogen fertilizers, sulphur fertilizer rates, and their interaction greatly increased sunflower crop growth (Ahmad et al.,2022), physiology (Ma et al., 2021), yield (Sadiq et al.,2021), oil contents (Sattar et al., 2021) and nitrogen use efficiency (El-Azeiz et al., 2021). Meanwhile, when PCU was used instead of CU, NUE, achene yield, and oil contents all increased by 16.0-17.2 percent, 16.5-17.0 percent, and 2.96-3.19 percent (Ghumman et al., 2021). SCU is the best among the slow release modulated forms of urea (Kumar *et al.* 2019), followed by NCU (Singh et al.,2021) and finally split application of urea (Dawar et al., 2024) as a nitrogen source for transplanted maize for increased growth and production. It was found that the slow release modified form of sulphur coated was more successful in increasing plant height, number of tillers/m, number of panicles/hills, number of grains/panicles, and dry matter accumulation/m^2 (Altri et al., 2023)

CONCLUSION

The research effectively distinguished *Brassica napus* genotypes with different levels of drought resistance by simulating drought stress using a 25% concentration of PEG-6000. Strong drought resistance was demonstrated by genotypes like Faisal Canola, which also maintained greater germination rates, increased seedling development, and enhanced physiological parameters including membrane integrity, leaf area, relative water content, and potassium content. Sulfur-coated urea (SCU) improved water management during droughts and nitrogen retention, which further improved these features. On the other hand, genotypes susceptible to drought exhibited notable declines in every attribute assessed, signifying their susceptibility to circumstances involving scarce water resources.

These results offer important information for creating *B. napus* varieties that are resistant to drought, which is essential for maintaining agricultural production in areas that are vulnerable to drought. In order to pinpoint the genes behind these genotypes' resistance, future studies should concentrate on the genetic underpinnings of drought tolerance. Furthermore, field tests conducted in actual environments might confirm the efficacy of SCU and the performance of certain genotypes that are drought-tolerant. In order to support sustainable agricultural production in the face of climate change, it may be possible to generate drought-tolerant cultivars more quickly by investigating molecular methods and modern breeding procedures.

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