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Screening of Beans (*Phaseolus vulgaris* L.) Genotypes for Drought Tolerance

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Abstract

Drought is a critical factor that limits crop yield and contributes to global food insecurity. In regions where beans are cultivated, 60% of the crop is vulnerable to drought, resulting in an 80% reduction in yield. This presents a growing threat to farming communities in Bhutan, where limited research on crop drought tolerance has been conducted. To address this issue, a study was undertaken to evaluate the drought tolerance of six bean genotypes: Orey serbu, Orey regtang, Orey brokchilu, Yadhipa orey, Kerongree orey, and Brokopali. The study employed a Completely Randomized Design (CRD) consisting of six treatments each with three replications. The genotypes were subjected to drought stress after 50% flowering until the onset of pod formation, with drought conditions maintained for 10 days during pod formation, followed by restored irrigation until harvest. Key parameters such as leaf area, root weight, shoot weight, as well as yield metrics including the number of pods and seed weight, were assessed at harvest. The findings indicated significant differences (p<.05) in all parameters measured under stressed versus non-stressed conditions. Water stress was found to adversely affect the growth and development of all bean genotypes. With performance as regards to Drought Susceptibility Index (DSI) which is a measure of yield stability (DSI = (1- Yd / Yw) / D. Brokpali, Yadhipa orey, and Orey serbu demonstrated the lowest values for the drought susceptibility index (DSI), suggesting a greater level of tolerance to drought for these specific genotypes.

Keywords: Drought susceptibility index, Drought stress, Tolerance

Introduction

Common beans (*Phaseolus vulgaris* L.) are consumed as a staple and low-cost protein source in underdeveloped countries where protein malnutrition is prevalent (Castaneda-Saucedo et al., 2009). Broughton et al (2003) stated that the genus *Phaseolus* originated from the Mesoamerican regions and comprises five domesticated species: *Phaseolus vulgaris* (common bean), *Phaseolus dumosus, Phaseolus coccineus* (runner beans), *Phaseolus acutifolius* (tepary beans), and *Phaseolus lunatus* (Lima bean). Dry beans are a staple food in Latin American, Eastern, and South African countries (Broughton et al., 2003). Similarly, beans are the source of dietary carbohydrates and micronutrients for more than 300 million people from Eastern Africa and Latin America (Welch et al., 2000; Beebe et al., 2013). India produced 6,75,188 metric tons [MT] of green beans, followed by Bangladesh (137495 MT) in 2023 (Food and Agriculture Organization of

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the United Nations [FAO], 2023). According to the Department of Agriculture [DoA], 2021), Bhutan experienced a notable decline in bean production, yielding 743.52 MT in 2021 compared to 3,168.51 MT in 2020. This significant reduction highlights concerns regarding bean sustainability and productivity in Bhutan. Bean production is adversely affected by both biotic and abiotic factors (Jaleel et al., 2009), and the major abiotic factor affecting crop production worldwide is water stress (Man, Bao & Han, 2011).

Water stress is ranked second after pests and diseases in reducing agricultural grains and legumes (Jaleel et al., 2009). Furthermore, 60% of the bean production in the world occurs in agricultural land prone to water deficit, and lack of irrigation system, and the dry periods may result in an 80% yield reduction (Kavar et al., 2008; Rosales et al., 2012). Drought stress lowers osmotic pressure, disrupts water potential in plant cells, and causes oxidative stress, leading to reduced photosynthesis and metabolic function (Mladenov et al., 2023). According to DoA (2021), the drought (4% of total households) and the insufficient irrigation supply (27% of total households) are emerging as a threat to agriculture in Bhutan. Beans are the most important, directly consumed food legume, and it is one of the most important cash crops after potatoes grown by every Bhutanese farmer. Almost every Bhutanese household consumes beans, and they remain as high demand in the market. However, with the changing weather conditions, including more frequent erratic rainfall and the risk of extended drought, there is a need for crops that perform well despite harsh weather (Wanders & Wada, 2015). Thus, this study examined the performance of traditional bean varieties that have been predominantly cultivated in rural areas for many years. The successful identification of local varieties exhibiting stress tolerance could enhance the diversity of stress-tolerant bean varieties in Bhutan, a topic that has not been published. The findings of this research is expected to contribute to maintaining production levels and meeting market demand in Bhutan and will also serve as a foundation for the future development of drought-tolerant varieties.

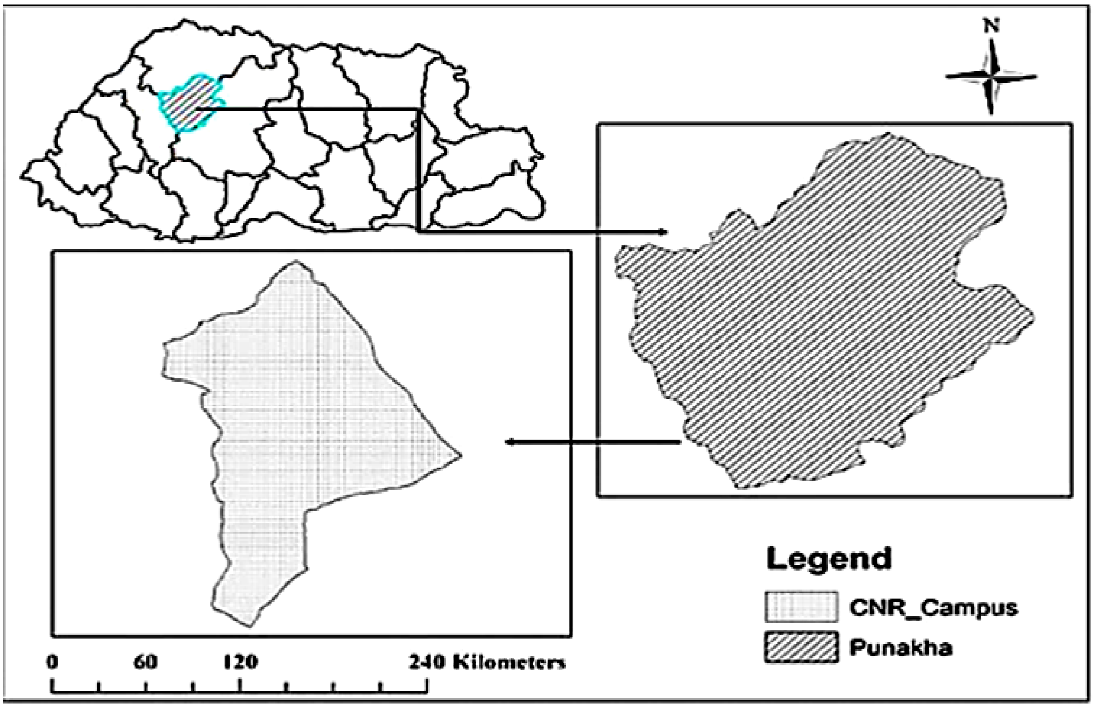

Figure 1: Study site, College of Natural Resources, Lobesa

Materials and Methods

Study site

The study was conducted in the greenhouse of the agriculture farm of the College of Natural Resources (CNR), Lobesa in July 2019. It is at an elevation of 1450 meters above sea level between 27° 30' 1'' N and 89° 52' 42'' E of Greenwich. It lies in the dry sub-tropical region and experiences hot and humid summers during the monsoon months of June, July, and August.

Genotypes used in the experiment

Six distinct locally grown bean genotypes were obtained from the Agricultural Research Development Center (ARDC) in Wengkhar. Collectively, these genotypes are commonly known by the "Sharshop" name as they are primarily cultivated in the eastern region of Bhutan.

Experimental design

The experiment was laid out in a completely randomized design (CRD) for this study. The six distinct genotypes were categorized into two treatment groups: water-stressed and non-stressed (control), with each group consisting of three replications, each comprising three plants. A total of 108 potted plants were used in the experiment, with 54 plants allocated for stressed treatments and 54 for non-stressed controls. Each pot contained three plants and was later thinned to two plants per pot for both stressed and non-stressed conditions. The spacing within rows was 30 cm, while the distance between rows was 40 cm (Alidu, 2018).

Table 1:Fifteen local beans genotypes

Entry no.	Place of collection	Accession name
6	Kerong	Orey Regtang
12	Gomchu	Yadhipa Orey
7	Kerong	Kerongree Orey
27	Nanong	Orey Serbu
15	Kanglung	Orey Brokchilu
25	Tsamang	Brokpali

Soil characteristics

The soil samples were collected and composite soil sample was air-dried inside tray. Foreign materials were removed and sieved under the 0.2 mm sieve. Later the samples were tested for soil pH, available nitrogen, potassium, phosphorus, soil organic carbon, and organic matter. The soil pH was determined by "Bray no 2 extraction" method and organic matter, organic carbon and available nitrogen were determined by "Dry combustion method". "Flame photometer" method was used to determine available potassium while "Olsen method" was used to determine the available phosphorus. The study did not use any fertilizers or organic manures during the entire experimental period.

Drought stress

The plants in the stress group (54 pots) were subjected to drought stress by withdrawing irrigation after the plants reached 50% flowering. This drought period lasted for 10 days, after which irrigation was resumed for two weeks then irrigation was withdrawn until the next tenth day (Alidu, 2018; Batieno et al., 2016; Abrokwah, 2016). The remaining 54 potted (control) plants received the water throughout the experiment.

Determination of Soil Field Capacity (FC)

The soil field capacity (FC) was determined using the formula (given below) recommended by Johnston and Askin (2005) and five pots filled with media (top soil) were taken and their average values were calculated.

Procedures:

% Moisture at Field Capacity

$$\frac{Weight\ of\ wet\ soil - weight\ of\ oven\ dry\ soil}{Weight\ of\ oven\ dry\ soil} \, x\ 100$$

Weight of the wet soil was recorded as per the method of Wehne, Balko and Ordon (2016) as by adding water into sample pots till saturation (Check with tensiometer) and keeping overnight until water is drained by gravity and then weighed.

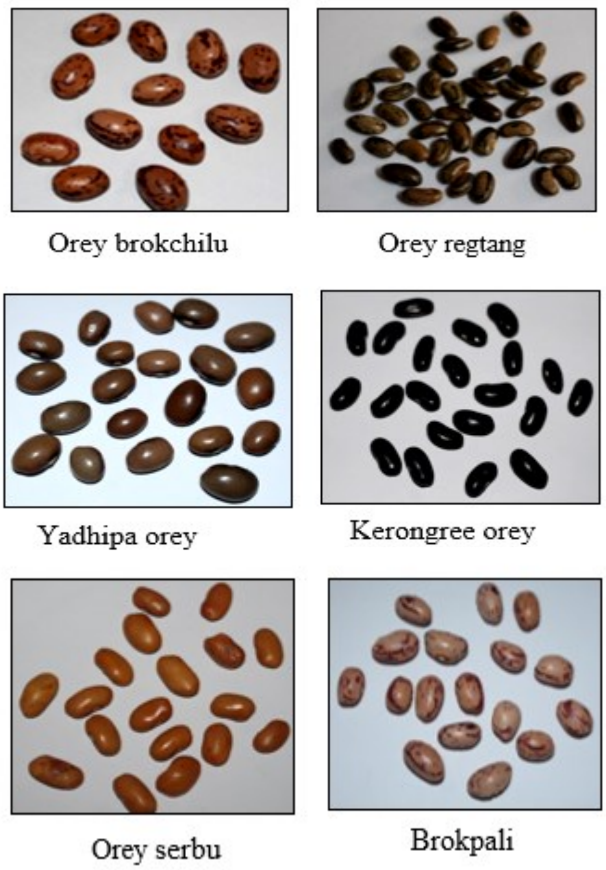

Figure 2: Local beans genotypes

% Moisture in Air Dry soil

To find moisture percent (%) in air dry soil, five samples of dry air soil were weighed and dried to constant weight at 105° C for 48 hours. Pots were kept inside the greenhouse for two days to dry the soil and weight of air dry soil was obtained by weighing the pots shown below:

$$\frac{\text{Weight of air dry soil} - \text{Weight of oven dry soil}}{\text{Weight of oven dry soil}} \times 100$$

Table 2: Soil characteristics

Soil property	Units	Values	Remarks
Available Potassium[K]	ppm	2,010	High
Available Phosphorus[P]	mg/kg	10	Medium
Total available Nitrogen[N]	%	0.07	Low
Organic Carbon[OC]	%	1.35	Moderately low
Organic Matter[OM]	%	2.32	High
pH		6.84	Neutral

Weight of Oven dry soil in a pot

Weight of oven dry soil to fill pot =

$$\frac{\text{Weight of air dry soil to fill pot} \times 100}{100 + \text{air dry \% moisture}}$$

Weight of soil and water at field capacity

$$\frac{[100 + \text{field capacity \% moisture}] \times \text{weight of oven dry soil}}{100}$$

Weight of water to add to each pot, of air dry soil (Water at field capacity)

Irrigation schedule

Pots were weighed keeping one day interval (Choudhury, 2010). Watering was done by weighing the pots and the amount of water applied was calculated by subtracting the weight of the pots at field capacity by the present weight. The soil in the pots was maintained at near water field capacity (FC) throughout the experiment (Batieno et al. 2016). Each pot before planting was watered to its field capacity.

Scoring and ranking of genotypes

The water-stress tolerance of six bean genotypes was ranked using the DSI values of each parameter. The scoring and the ranking were based on a scale where the least susceptible genotypes scored 1, and the most susceptible scored 6. A Drought Susceptibility Index (DSI) value of less than 1.0 indicates tolerance to drought, while a DSI of 0.0 indicates maximum possible drought tolerance with no effect on yield (Abrokwah, 2016). The negative value in the scoring indicates greater tolerance and vice versa.

Drought susceptibility indices (DSI)

The drought susceptibility index (DSI) was calculated using the formula of Abrokwah (2016) to help determine the degree of susceptibility to the stress condition.

$$DSI = 1 - Y_d/Y_w \div D$$

$$D = 1 - \frac{Mean\ yield\ under\ drought\ stress\ (Grand\ mean)}{\text{Mean yield under non} - \text{stressed (Grand mean)}}$$

Where:

D = drought intensity

Y_d = Mean yield under drought conditions (Average yield of genotypes)

Y_w = Mean yield under well-watered conditions (Average yield of genotypes)

Data collection

The weather data were recorded using a data-logger, indicating an average temperature during the month of August 38.38^{0}C and the temperature dropped to 26.82^{0}C in September and further to 22.72^{0}C in the month of October. The recorded average relative humidity (RH%) during the time of experiment was 51.7% in August, 59.52% in September and 50.51% in the month of October. Growth and yield attribute data were gathered at physiological maturity, defined as the period when 75% to 90% of pods lose their green pigmentation (Vallejo & Kelly, 1998).

Leaf area(dm^2)

To maintain uniformity first leaf from below the sample plants was taken and leaf area was determined with the help of Stickler's linear measurement method (Stickler & Pauli, 1961) as given below: leaf area per plant was calculated and expressed in dm^2. Average leaf area was recorded.

Leaf area (dm^2) = L x B x 0.747

L = length of leaf

B = breadth of leaf

0.747 = correction factor

Number of pods per plant

The pods from two randomly selected plants were removed, counted, and divided by two to obtain the average number of pods per plant.

Shoot dry weight and root dry weight (g/plant)

Two plants were randomly sampled from each row and carefully uprooted. The shoot system was separated from the root system and placed in labeled envelopes. They were oven-dried at 60°C for 72 hours and then weighed (Alidu, 2018).

Seed weight (g/plant)

The seeds from the two sample plants were collected, and the average seed weight was calculated per gram per plant.

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Data Analysis

Data were subjected to an ANOVA (Analysis of variance) using Statistics version 8. Individual means of water-stressed genotypes were compared to their corresponding non-stressed in pairwise comparison analyses (t-test) and the Least Significant Difference (LSD) was used to determine differences in treatment means at a 5% probability level.

Results and Discussion

Effect of drought stress on leaf area (dm2)

A significant difference in leaf area was identified between the treatment groups (stressed and non-stressed) [$F(1, 22) = 52.96$, $p < .05$] and among the various genotypes [$F(5, 22) = 10.42$, $p < .05$]. Under water-stressed conditions, most genotypes exhibited a reduced leaf area compared to those under non-stressed conditions (Figure 3). The decrease in leaf area is regarded as a strategic mechanism to minimize the evaporative surface area (Narejo et al., 2018). The genotypes Orey Serbu (M = 0.55 dm², SD ± 0.05) and Orey Regtang (M = 0.55 dm², SD ± 0.03) displayed significantly larger leaf areas under water stress. However, no significant differences ($p > .05$) were observed among the genotypes Yadhipa Orey, Orey Brokchilu, and Brokpali under stress condition. The reduction in leaf area under stress may be attributed to factors such as wilting, leaf shedding, curling, stomatal closure, and diminished cell enlargement.

During the onset of water stress, it inhibits cell elongation in the leaf, and the lower leaf area leads to less water uptake from the soil, and transpiration is reduced (Fathi & Tari, 2016). Under the non-water stressed condition genotypes, Orey serbu had recorded significantly higher in leaf area (M = 0.75 dm2, SD ± 0.01). This result is consistent with the observations made by Samwel (2008) and Choudhury et al. (2010), who found that rice cultivated under water-deficit conditions had a reduced leaf area compared to plants grown in optimal conditions. Additionally, Severino et al. (2004) posited that leaf area is closely linked to plant metabolism, dry matter production, and overall yield.

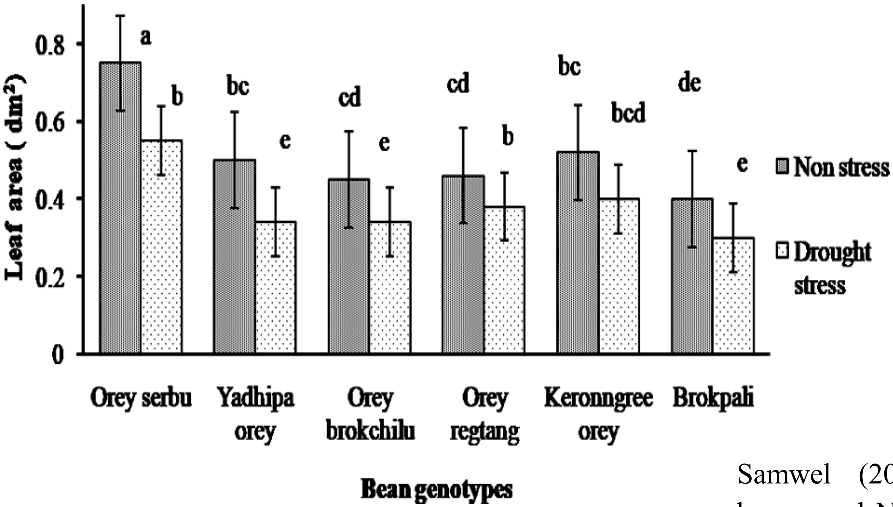

Figure 3: Average Leaf Area (dm^2 per plant) in both stressed and non-stressed conditions.

Effect of drought stress on pod number

There was a notable difference in the number of pods per plant, influenced by both treatment and genotype groups, as demonstrated by the analyses [F(1, 22) = 64.61, P < .05] and [F(5, 22) = 8.67, P < .05]. The study highlighted that varietal differences in pod number were affected by the stress conditions imposed (Figure 4). Under water-stressed conditions, the number of pods per plant decreased in comparison to non-stressed conditions. The genotype Yadhipa Orey exhibited the highest average number of pods (M = 5.25, SD ± 0.33). The reduction in floral part formation may have played a role in the diminished number of pods per plant. Conversely, under non-water-stressed conditions, the genotype Orey Regtang showed the highest average number of pods (M = 7.39, SD ± 0.98).

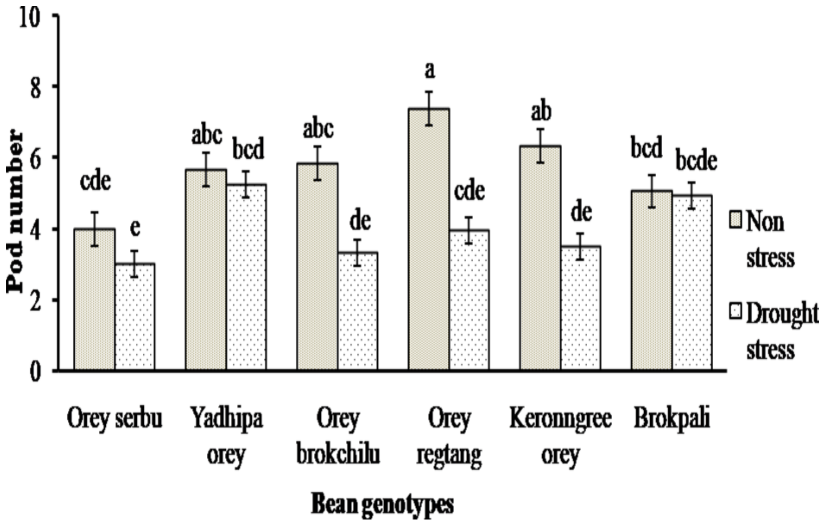

Figure 4: Average number of pods (per plant) in both stressed and non-stressed conditions.

This study is consistent with the findings of Castaneda-Saucedo et al. (2009), who demonstrated that high moisture stress during the reproductive stage can lead to floral abortion, thereby resulting in reduced yields. Additional research by Batieno et al. (2016) and Singh (1995) also indicates that water stress experienced during the flowering and pod-setting phases contributes to the abortion of flowers and pods. Moreover, the results align with the work of Samwel (2008), Barrios, Hoogenboom, and Nesmith (2005), and Martínz et al. (2007), all of whom reported a decrease in pod numbers with irrigation conducted at 21-day intervals. The reproductive stage of the beans is particularly vulnerable to drought stress (Nielsen & Nelson, 1998).

Effect of drought stress on seed weight (g/plant)

There was a highly significant difference in seed weight, both between treatments and within the genotype groups [F (1, 22) = 597.64, p < .05] [F (5, 22) = 35.76, p < .05]. Under stress conditions, both seed weight and yield experienced a decline compared to non-stress conditions [Figure 5]. Notably, the Brokpali genotype recorded the highest seed weight under stress (M = 5.94g, SD ± 0.59). This decrease in seed weight and yield during water stress is likely due to reduced carbohydrate assimilation, which may have led to a lower number of pods. In non-water stress conditions, Brokpali achieved the highest average seed weight of (M = 14.51g, SD ± 1.51). The reduction in legume seed yield under stress can primarily be attributed to a decrease in the number of pods per plant (Lopez et al., 1996; Muchow et al., 1992; Muchow, 1985; Batieno et al., 2016). Overall, the negative impact of drought on pod development and grain filling must have contributed to the reductions in seed yield and pod numbers (Chiulele, 2010).

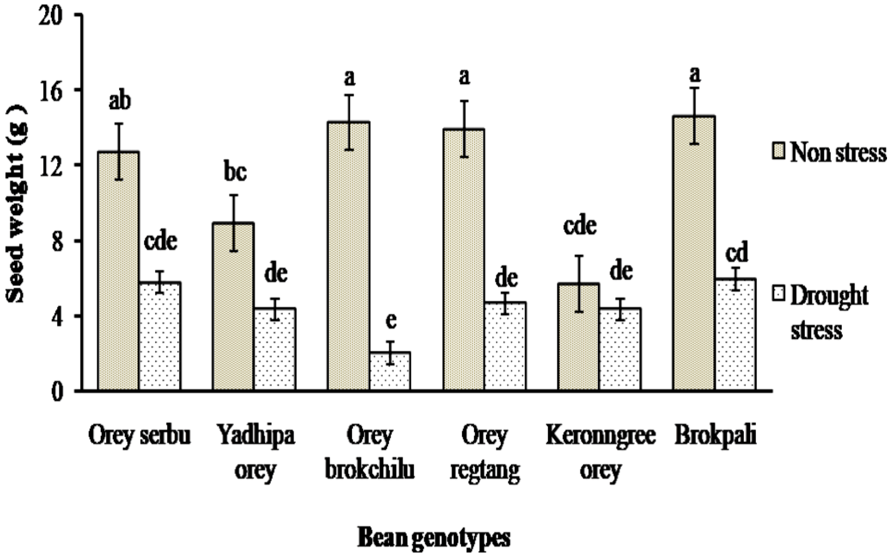

Figure 5: Average Seed weight (g/plant) in both stressed and non-stressed conditions

The yield reductions in common beans were reported to range from 58% to 87% when water stress occurs during the reproductive stage (Tekle & Alemu, 2016; Nam, Chauhan & Johansen, 2001; Soureshjani et al. 2019; Ahmad, Selim, Aldrfasi, & Afzal 2015; and Abrokwah 2016). Furthermore, significant seed yield reductions due to water stress have been documented for the black gram and green gram (Tripurari & Yadav, 1990; Kalim, 2013), as well as soybean (Liu, Andersen, & Jensen, 2003) and Phaseolus vulgaris (Omae et al., 2005; Islam et al., 2004; Choudhury et al., 2010). The decline in seed yield in common beans under stress was largely due to reduced photosynthate assimilation and impaired carbohydrate partitioning to the developing grains because of drought conditions (Perea-Munoz et al., 2007; Rezene, Gebeyehu, & Zelleke, 2011; Asfaw et al., 2012).

Effect of drought stress on shoot dry weight (g/plant)

The study identified a significant difference in shoot weight between the treatment group [F (1, 22) = 40.15, p < .05] and the genotype group [F (5, 22) = 13.81, p < .05]. Under water stress conditions, the dry weight of plant shoots decreased significantly compared to non-stress conditions (Figure 4). This decline could likely be due to reduced photosynthate assimilation and limited water absorption by the roots. Notably, Orey Serbu exhibited the highest shoot weight under water stress, with a mean of 6.91 g (SD ± 0.14). Conversely, in non-water stress conditions, Brokpali recorded the highest shoot weight at a mean of 14.61 g (SD ± 0.29). Similar outcomes have been reported by Okçu, Kaya, and Atak (2005) and Bibi et al. (2010), highlighting that shoot growth tends to diminish more significantly than root growth under stress. According to Tekle and Alemu (2016), both shoots and roots are the most impacted parts of plants and play vital roles in drought adaptation at the morphological level.

Drought stress notably impairs stem growth and the ability to capture solar radiation (Abrokwah, 2016). During such stress conditions, the Orey serbu, Brokpali, and Orey regtang genotypes demonstrated significantly higher shoot dry weight compared to other genotypes. According to Brdar-Jokanović et al. (2014), there exists a strong positive correlation (r = 0.53**) between shoot and root dry weight under optimal irrigation. However, this correlation does not persist under drought stress (r = 0.11), likely due to genotypic variations in root adaptation to stress.

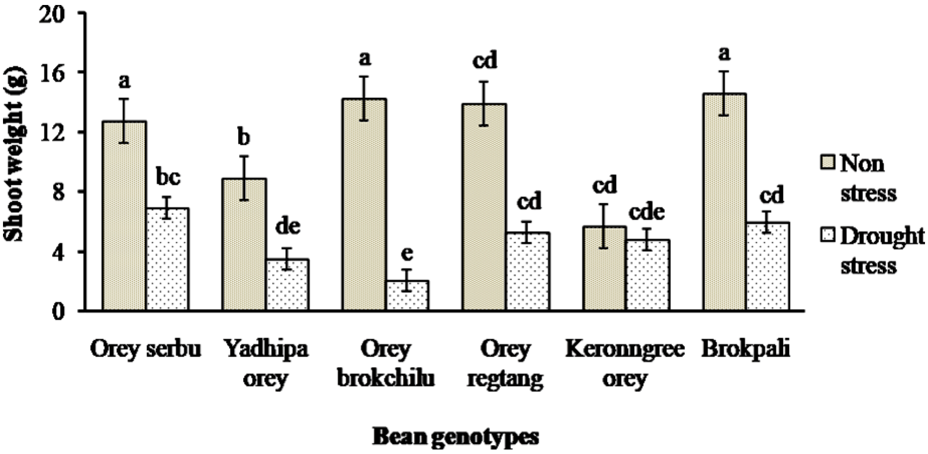

Figure 6: Average Shoot weight (g/plant) in both stressed and non-stressed conditions.

Effect of drought stress on root dry weight (g/plant)

Significant differences ($p < .05$) were noted in root weight between the treatment and genotype groups, with values of [$F(1, 22) = 135.47$, $p < .05$] and [$F(5, 22) = 6.36$, $p < .05$]. Under water-stressed conditions, root dry weight generally decreased compared to non-stressed conditions (Figure 7). This reduction in root dry weight can be attributed to diminished root proliferation in stressful environments, as previous research suggests that plants subjected to water deficit conditions generally display lower root biomass (Nleya, Slinkard & Vandenberg, 2001). However, the Brokpali genotype exhibited the highest root weight under water stress, with a mean of 1.84 g and a standard deviation of ± 0.36. The higher root weight of the Brokpali genotype indicates a greater ability for the plant's roots to extend further and thrive under stressed conditions, making it suitable for drought-prone areas. In contrast, under non-water-stressed conditions, the Keronngree orey genotype exhibited the highest root weight, with a mean of 2.9 g (SD ± 0.23). This suggests that while it performed well in favorable conditions, it did not thrive under water-stressed conditions.

In contrast, the increase in root weight diameter was reported in a legume *Trifolium repens* due to drought stress. Moreover, the study revealed that roots have a greater drought response compared to shoots probably due to the roots being involved in water uptake and directly in contact with the soil (Reinelt et al., 2022). Drought stress can significantly affect the

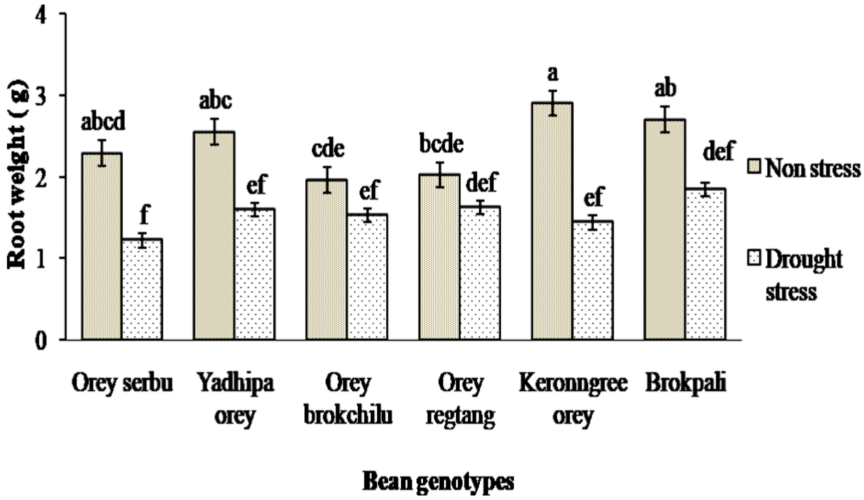

Figure 7: Average Root weight (g/plant) in both stressed and non-stressed conditions.

Table 3: Scoring and Ranking of Genotypes

Genotypes	LA	SHW	RW	SW	PN	RL	Rank Sum	Rank
Orey serbu	2	1	5	3	3	5	19	2nd
Yadhipa orey	1	4	4	2	2	6	19	2nd
Orey brokchilu	3	6	2	6	4	4	25	5th
Orey regtang	6	5	1	5	6	1	24	4th
Kerongree orey	5	2	6	1	5	2	21	3rd
Brokpali	4	3	3	4	1	3	18	1st

LA: Leaf area, SHW: Shoot weight, RW: Root weight, PN: Pod number, and RL: Root length

yield components and biomass accumulation in bean plants (Munoz-Perea et al., 2006). Nevertheless, certain genotypes, such as Brokpali (1.84 g), Orey regtang (1.62 g), and Yadhipa orey (1.60 g), demonstrated superior performance under such stress conditions. This indicates that these genotypes may have enhanced efficiency in acquiring soil water compared to others. Dhole and Reddy (2010) noted that as water potential decreases, the number of roots diminishes, which directly impacts root biomass (Ranawake et al., 2001). Important root traits—including biomass, length, density, and depth—serve as key drought avoidance characteristics, significantly contributing to yield in environments experiencing terminal drought (Kavar et al., 2008).

Drought intensity (D) and Drought susceptibility Index (DSI)

The DSI for leaf area ranged from -0.27 to 0.27, for shoot weight from -0.13 to 1.45, for root weight from 0.56 to 1.43, for seed weight from 0.38 to 1.41, and for pod number from -0.41 to 0.22. A Drought Susceptibility Index (DSI) value of less than 1.0 indicates tolerance to drought, while a DSI of 0.0 indicates maximum possible drought tolerance with no effect on yield.

Scoring and ranking of genotypes based on drought susceptibility indices

The ranking was based on a scale where the least susceptible genotypes scored 1, and the most susceptible scored 6 (Table 4). The lesser the rank sum of all the genotypes, highest the rank indicating higher tolerance. The genotypes Brokpali (1st), Orey serbu, and Yadhipa orey(2nd) scored the lowest DSI, indicating greater drought tolerance. On the other hand, Orey brokchilu (5th), Orey regtang (4th), and Kerongree orey(3rd), indicated they were more susceptible to drought conditions.

Table 4: Drought susceptibility index (DSI)

	DSI				
Genotypes	LA	SHW	RW	SW	PN
Orey serbu	-0.18	-0.13	1.33	0.89	-0.09
Yadhipa orey	-0.27	1.03	1.06	0.84	-0.34
Orey brokchilu	-0.15	1.45	0.62	1.41	0.17
Orey regtang	0.27	1.05	0.56	1.09	0.22
Kerongree orey	0.058	0.27	1.43	0.38	0.19
Brokpali	0.034	1.01	0.91	0.97	-0.41

LA: Leaf area, SHW: Shoot weight, RW: Root weight, SW: Seed weight, and PN: Pod number

Conclusion

The current study indicates that Brokpali (1st), Orey serbu (2nd), and Yadhipa orey (2nd) are likely to perform well under limited water supply conditions, given their low drought susceptibility index observed in greenhouse settings. In contrast, Orey regtang (4th), Kerongree orey (3rd), and Orey brokchilu (5th) demonstrated the least resistance to drought. Notably, drought stress significantly diminished leaf area, pod numbers, and seed weight per plant compared to conditions without stress. This adverse condition impeded proper growth and development, ultimately affecting overall plant yield. Nonetheless, further research should investigate additional parameters such as yield production outside of greenhouse environments, employing a more precise methodology to enhance our understanding of bean variety performance under water stress conditions.

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