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Original Research Paper

Detection of onion responses to water stress using physiological and biochemical parameters

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ABSTRACT

Onion, an important vegetable crop valued for its culinary and medicinal properties, is highly sensitive to water stress, which significantly affects its growth, yield, and quality. In this study, the response of two onion genotypes to water stress was evaluated using a combination of physiological and biochemical parameters, including thermal imaging. The plants were maintained at five different field capacity levels by gradually achieving the desired stress conditions using a gravimetric approach. The results indicated that water stress caused a marked decline in growth parameters, relative water content, and chlorophyll content, whereas, it induced an increase in malondialdehyde content, proline content and the canopy temperature of leaf tissue in both the genotypes. However, 'Bhima Shakti' performed better under water stress than 'Bhima Shweta'. Drought stress at 60% field capacity caused a significant reduction in relative water content (54.03%), along with increased lipid peroxidation (56.1 nanomoles/g) and a higher canopy temperature (38.0°C) in the susceptible genotype 'Bhima Shweta'. In contrast, the drought-tolerant genotype 'Bhima Shakti' exhibited resilience, maintaining higher relative water content (62.25%), lower lipid peroxidation (42.1 nanomoles/g), and a cooler canopy temperature (36.5°C) under the same level of stress. These findings suggest that relative water content, lipid peroxidation, and canopy temperature could be useful screening tools for assessing water stress tolerance in onion.

Keywords: *Allium cepa*, canopy temperature, drought, lipid peroxidation, proline

INTRODUCTION

The result of global climate change on crop production has become as a major research priority in the recent scenario. The need to secure food supplies for the future during this imminent global climate change scenario, has becomes crucial to understand the consequences of climate change on the productivity of important crops. Plants have developed potent responses at the morphological and cellular levels allowing them to adapt to unfavourable environmental conditions. Drought stress is the key environmental stress arising from low rainfall, high light intensity and temperature (Azadi et al., 2018). Water deficit stress is a major limiting agent of crop production worldwide. Under changing climate scenario there is a continuous demand for genetically new drought-resistant crops (Snowdon et al., 2021). One way to improve the drought resistance of cultivated crops is to find genotypes that are well adapted to such harsh conditions (Gedam et al., 2021). Drought stress affects numerous metabolic and physiological processes in plants including growth reduction, reduction in tissue water content, reduction in the chlorophyll content, production of free radicals, changes in fluorescence parameters etc. (Gurumurthy et al., 2019; Nemeskéri & Helyes, 2019). Drought stress, at the cellular level, enhances the generation of active oxygen species, such as superoxide radicals, hydrogen peroxide and hydroxyl radicals. Plants control these active oxygen species through different enzymatic and non-enzymatic scavenging mechanisms. Osmoregulation is also important for tackling harmful effects caused by free radicals under abiotic stress conditions (Mahmood et al., 2020). Through accumulation of different osmolytes, such as soluble carbohydrates, proteins, free amino acids, glycine betaine, and proline, plants tend to increase the osmotic potential at the cellular level (Blum, 2017). Drought stress also regulates the physiological responses of plants under stress conditions. In drought stress, variation in physiological responses indicate sensitivity and resistibility of the crop plants and thus help in phenotyping (Ahmed et al., 2020). One important phenotyping technique in plant physiology is use of IR thermal sensing, which is non-destructive, advanced, accurate, and less time consuming and is being used for the screening of stress-tolerant plants under abiotic stress environments (Taria et al., 2022). Here, leaf temperature is an indicator of the internal water status of the plant under stress conditions. Thus, IR thermography could be a good non-destructive method for the rapid screening of genotypes under drought stress conditions (Taria et al., 2022). Biochemical and physiological parameters are also crucial for screening genotypes for their tolerance and susceptibility (Gedam et al., 2021).

Onion (*Allium cepa* L.), is an economically important vegetable crop with the highest foreign exchange earning capacity among fruits and vegetables (Ardeshna et al., 2014). It is a valuable crop used widely in culinary preparations (Singh & Gopal, 2019). It is an important component of our daily diet and is cultivated worldwide. In the whole world, India ranks first in area and second in production (http://agricoop.gov.in/). Though onion is grown in huge area, productivity is still less than in other countries (Tripathi et al., 2017). The major reason for its low productivity is mainly the environmental constraints *viz*., biotic and abiotic stress. Being a shallow rooted-crop, water deficit stress is a big issue for onion growth and development (Pelter et al., 2004). It was observed that water deficit stress leads to reduction in marketable yield at all the stages of plant growth (Pelter et al., 2004). Growth and productivity of plants depend on environmental conditions, and drought is a worldwide concern, and identification of drought stress adaptive mechanisms is essential for crop improvement programs. Genotypic diversity can be exploited for development and identification of tolerant genotypes to combat water deficit issue (Gedam et al., 2021). In India, onion is cultivated in three seasons monsoon, late-monsoon and spring contributing 20%, 20% and 60%, respectively. Spring harvest, the major contributor, is susceptible to water deficit stress as it is grown before the rainy season. Despite of huge potential of onion production, low water availability to the crop during spring causes a significant reduction in productivity. Thorough studies are needed to identify important physiological and biochemical parameters that impart plant tolerance. Hence, this study was planned to evaluate different physiological and biochemical parameters at different levels of stress, which led to yield reduction under stress conditions.

MATERIALS AND METHODS

Imposition of water deficit stress

The experiment was conducted at the pot culture area of ICAR-Directorate of Onion and Garlic Research (DOGR), Rajgurunagar, Pune, Maharastra, India (32°N and 73.51°E at 553.8 m above mean sea level). Two onion genotypes, 'Bhima Shakti' and 'Bhima Shweta' were selected for this study. Seeds were sown in the field, and seedlings were transplanted to pots after 45 days. Plastic pot measuring 30 cm in diameter, containing 12 kg soil and farmyard manure in 3:1 ratio were used in this study. Treatments were applied after four weeks of transplanting which is at the bulbing stage. The experiment was carried out in a CRD design with five replications. Five different water levels were maintained by the gravimetric method (Campbell & Mulla, 1990): 100, 80, 60, 40, and 20% of field capacity (Fig. 1). The water-holding capacity of the soil mixture was determined, and the required volume of water to reach 100, 80, 60, 40, and 20 % field capacity (FC) was calculated. The water transpired from each pot was determined by weighing the pots daily, gradually adjusting the soil moisture regime until it reached different field capacities. Treatments were continued for four weeks. All measurements were carried out after 28 days when the plants in severe stress started to wilt.

Physiological parameters

To access morphological parameters, plant fresh weight (g), plant dry weight (g), and plant height (cm) were recorded for control and drought-stressed plants. Plants at 100% field capacity were the control, while, those at 60%, 40%, and 20% were the stressed plants. Plants were carefully uprooted after 28 days of treatment to measure height, fresh weight and dry weight. Dry biomass was measured after oven-drying at 70°C for 72 h. All the variables measures were recorded as the mean value based on the fifteen plants per individual genotype per treatment.

The physiological and biochemical parameters were studied in the fourth leaf. Leaf relative water content (RWC) was estimated by recording the fresh and turgid weight, and dry weight of a fully developed, young leaf for both control and drought-stressed treatments. The leaf relative water content was calculated using the following formula (Kramer, 1988):

RWC = [(FW –DW)/(TW– DW)] × 100

where, FW is the fresh weight, DW is the dry weight, and TW is the turgid weight.

To differentiate the responses to varying levels of soil moisture stress, thermal images were captured by Jenoptik Hr 575 infrared camera (InfraTec, GmbH, Germany) that operates in the spectral range of 7.5–14 µm with a spatial resolution of 768 × 576 pixels. To estimate the canopy temperature of each plant (treatment), thermal images were processed using the Software package IRBIS® 3 software (InfraTec, GmbH, Germany).

Biochemical parameters

For total chlorophyll estimation 50 mg leaf material was extracted by 80% acetone and the absorption rate of samples was measured at wavelengths of 663 and 645 nm by using a spectrophotometer. Chlorophyll content was calculated according to Arhon (1949).

Total chlorophyll (mg/g) = $[(8.02 \times A_{663}) + (20.2 \times A_{645})] \times [V/(1000 \times W)]$

The level of lipid peroxidation was measured in terms of thiobarbituric acid-reactive substances (TBARS) content by measuring the concentration of malondialdehyde (MDA) (Heath & Packer, 1968). According to this method, 0.5 g of fresh leaf sample was homogenized in 10 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 × g for 15 min. Supernatant (1 mL) was transferred to a new tube and 4.0 mL of 0.5 % (w/v) thiobarbituric acid (TBA) in 20% TCA was added. This mixture was heated at 95°C for 30 min and then cooled in an ice bath. After centrifugation at 10,000 × g for 10 min the absorbance of the supernatant was recorded at 532 nm. The TBARS content was calculated according to its extinction coefficient $\varepsilon = 155\ mM^{-1}\ cm^{-1}$. The values for non-specific absorbance at 600 nm were subtracted.

Proline content of leaves was determined using the method described by Bates et al. (1973). About 0.5 g leaf sample was homogenized in 10 mL of 3% aqueous sulphosalicylic acid and the homogenate was centrifuged at 10,000 × g for 10 min. The 2 mL supernatant was boiled at 100 °C for 1 h in a water bath with 2 mL of acid ninhydrin and 2 mL of glacial acetic acid. After chilling in at ice bath, 4 mL of toluene was added to the reaction mixture and mixed thoroughly. The absorbance of the part with toluene was recorded at 520 nm. Proline concentration was expressed in mg/100 g.

Data analysis

Samples for biochemical estimations were collected in 5 replicates (from 5 pots). The design of the experiment was CRD, and data were analyzed for analysis of variance (ANOVA) using LSD test at the significance level of 0.05 in SAS 9.3 programming environment.

RESULTS AND DISCUSSION

This study was conducted to evaluate the effect of moderate and severe drought stress treatments on onion genotypes, and their morphological, physiological, and biochemical effects were examined. The plants showed stunted growth and severe leaf

Fig. 1 : Onion genotypes at different field capacities (FC %): 100, 80, 60, 40 20. (A) Bhima Shakti and (B) Bhima Shweta

yellowing as the soil moisture levels were depleted (Fig. 1). The results of the mean comparison of the studied traits for both genotypes indicate that there is a significant effect ($P < 0.05$) in most of the traits due to water stress conditions.

Plant height, fresh weight and dry weight

The results of the comparison of mean values show that plant height was decreased by applying drought stress in onion genotypes (Fig. 2). Plant height is the major character significantly affected due to drought. The genotype 'Bhima Shakti' exhibited the highest plant height (48.71 cm) at 100% FC, while 'Bhima Shweta' showed the lowest height (17.18 cm) at 20% FC. The data showed that at a moderate stress level (60% FC), the percentage decrease in plant height was 11.6% in 'Bhima Shakti' and 15.53% in 'Bhima Shweta,' while, at a severe stress level (40% FC), it was 47% in 'Bhima Shakti' and 49% in 'Bhima Shweta'.

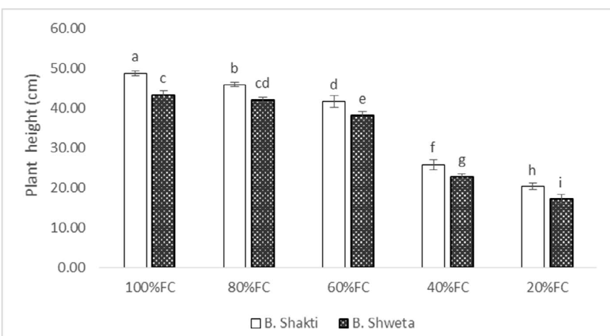


Fig. 2 : Effect of water stress on plant height of onion genotypes

The results of the study revealed a significant decrease in both fresh weight and dry weight under water stress for both onion genotypes (Fig. 3 & 4). The highest and lowest fresh weight and dry weight were observed in the control group (100% FC) and the severe stress group (20% FC), respectively. The fresh weight decreased by 38%, 80%, 93%, and 94% in 'Bhima Shakti' and by 34%, 75%, 89%, and 91% in 'Bhima Shweta' at 80%, 60%, 40%, and 20% of FC, respectively, compared to well-watered plants (100% FC). Similarly, the dry weight decreased by 32%, 79%, 91%, and 92% in 'Bhima Shakti' and by 22%, 72%, 86%, and 85% in 'Bhima Shweta' at 80%, 60%, 40%, and 20% of FC, respectively, compared to well-watered control plants at 100% FC. Dry weight is a significant parameter directly contributing to yield, and water stress can severely impact both dry weight and yield. These findings are consistent with previous research, which demonstrated that drought stress significantly reduces plant height and weight, leading to yield reduction (Gedam et al., 2021). Overall, these results suggest that water stress can significantly impact the growth and yield of onion genotypes, highlighting the importance of developing drought-tolerant onion varieties to ensure sustainable onion production in water-limited environments.

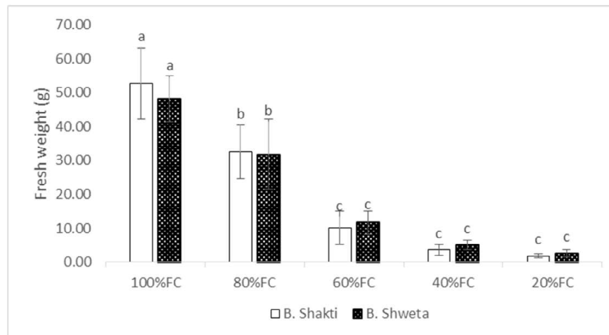


Fig. 3 : Effect of water stress on fresh weight of onion genotypes

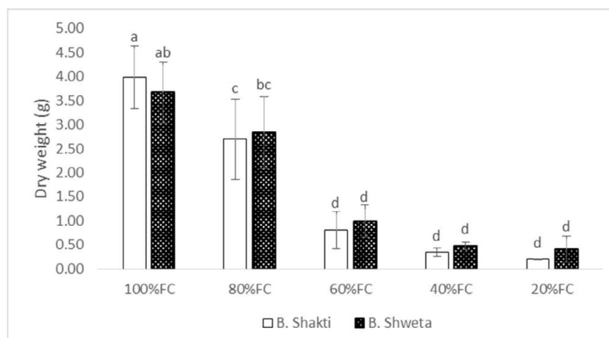


Fig. 4 : Effect of water stress on dry weight of onion genotypes

Relative water content (RWC)

Relative water content is considered a useful indicator of plant water status and is used to assess dehydration tolerance. It indicates the amount of water that the plant requires to reach full saturation. In this study, both genotypes exhibited a decrease in RWC in response to low water levels. However, 'Bhima Shakti' maintained a higher RWC (62.25%) compared to 'Bhima Shweta' (54.03%) under moderate stress conditions at 60% field capacity (Fig. 5). It has been reported that the reduction in leaf RWC depends on the tolerance level of onion genotypes (Ghodke et al., 2020). There was no significant difference in RWC at fully saturated conditions (100% FC) and at very severe stress (40 and 20% FC). The ability to retain water is vital for a plant's survival under water stress, as low levels of leaf water status can affect stomatal closure and photosynthesis rate, ultimately leading to reduced growth and yield (Ghotbi-Ravandi et al., 2014). Therefore, a genotype with better water retention capacity would have better adaptation under stress conditions. Overall, RWC is a critical parameter to consider when evaluating a plant's response to water stress, as it can indicate its ability to tolerate dehydration and adapt to changing environmental conditions.

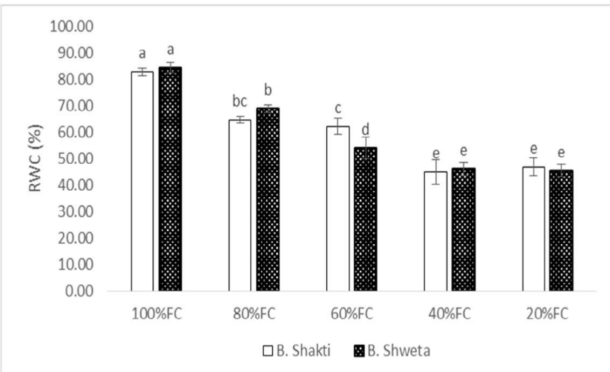


Fig. 5 : Effect of water stress on relative water content (RWC) content of onion genotypes

Chlorophyll content

Chlorophyll content is a crucial indicator of plant health and photosynthetic activity. Both the genotypes showed a significant decrease in chlorophyll content at different stress levels (60, 40 and 20% FC) compared to the control condition (100% FC) (Fig. 6). Although, there was no significant varietal variation between the two genotypes, the decrease in chlorophyll content was notable. Specifically, 'Bhima Shakti' and 'Bhima Shweta' exhibited a reduction of up to 84% and 77%, respectively, at 20% FC compared to the control (100% FC). A decrease in chlorophyll content under water stress could be credited to the breakdown rate of chlorophyll or halted biosynthesis due to reduction in the activity of chlorophyll biosynthesis enzymes or changes in thylakoid membrane structure (Bussis et al., 1998). Water stress can cause oxidative stress due to the inhibition of the photosynthetic activity and imbalance between the light capture and its utilization (Foyer & Noctor, 2000). Chlorophyll is a crucial photosynthetic pigment that contributes to plant growth and development, and ultimately yield, making it an important indicator of plant health under water stress conditions (Gurumurthy et al., 2019).

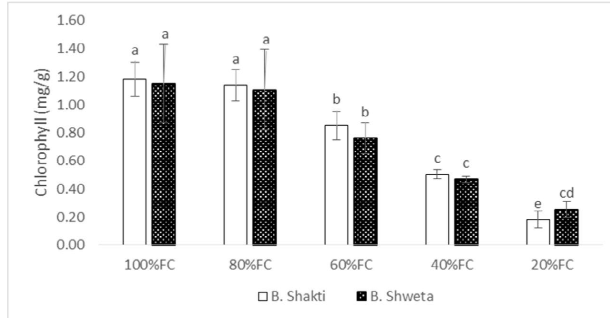


Fig. 6 : Effect of water stress on chlorophyll content of onion genotypes

Malondialdehyde (MDA) content

Water stress results in the overproduction of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radical, hydroxyl radical, etc. (Nawaz et al., 2020). ROS produced within plant tissue by lipid peroxidation damages the membrane by the formation of malondialdehyde (MDA). It is one of the important indicators of oxidative stress. As shown in Fig. 7, MDA content significantly increases under water stress, with the lowest value observed in non-stressed plants at 100% FC. It is worth noting that while MDA content increased with increasing stress levels, it decreased under severe stress conditions. The MDA content in 'Bhima Shakti' was 42.1 and 28.6 nanomoles/g, while in 'Bhima Shweta,' it was 56.1 and 35.0 nanomoles/g at 60% and 40% FC, respectively. MDA levels were found to be higher in 'Bhima Shweta' compared to 'Bhima Shakti'. The higher MDA content observed in 'Bhima Shweta' as compared to 'Bhima Shakti' suggests that 'Bhima Shweta' may be more susceptible to oxidative stress under water stress conditions. MDA levels indicates lipid peroxidation, which is mediated by high levels of ROS. A low MDA level indicates less damage by ROS and increased tolerance of the plant to water stress (Gurumurthy et al., 2019).

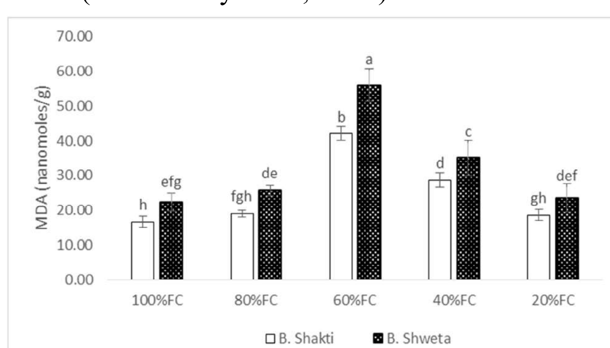


Fig. 7 : Effect of water stress on MDA content of onion genotypes

Proline content

Proline accumulation is a common strategy employed by plants to mitigate the effects of water-deficit stress. Proline content is often considered an index of moisture stress tolerance in plants. The proline content of leaves increased signiûcantly with the progression of water stress in both the genotypes (Fig. 8). However, the magnitudes of proline were more in 'Bhima Shweta' than in 'Bhima Shakti'. The increase in proline content was more prominent at 60% and 40% FC as compared 100%, 80%, and 20% FC levels. It increased by 155.64% and 142.75% in 'Bhima Shweta' and by 92.81% and 94.96% in 'Bhima Shakti' at 60% and 40% FC, respectively, compared to well-watered plants at 100% FC. Greater proline accumulation has been reported in drought-tolerant onion genotypes than in susceptible ones, indicating the importance of proline in drought tolerance (Ghodke et al., 2020). The accumulation of proline is a result of osmotic adjustment, which helps plants to synthesize and accumulate osmolytes or osmoprotectants to cope with osmotic stress (Blum, 2017). Overall, proline accumulation is an important mechanism that helps plants to adapt to water stress by maintaining cellular homeostasis and improving water use efficiency.

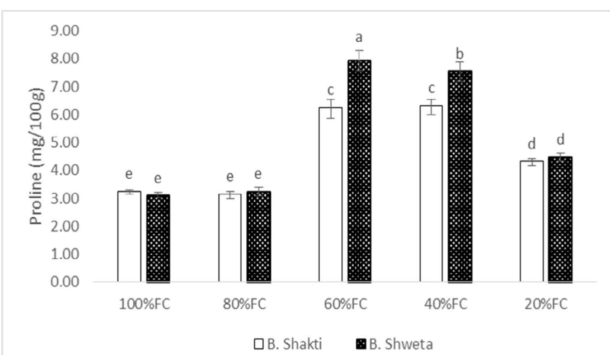


Fig. 8 : Effect of water stress on proline content of onion genotypes

Canopy temperature

Canopy temperature is regulated by a variety of factors, including transpiration rate, water level, and atmospheric temperature. Under water stress conditions, stomata closure causes a decrease in transpiration leading to an increase in the canopy temperature of plants (Nemeskéri & Helyes, 2019). The major task of transpiration is to keep the temperature of plants at an amicable level for different physiological processes. There is an inverse relationship between transpiration and canopy temperature (Nemeskéri & Helyes, 2019). The variation in canopy temperature among these two genotypes at different field capacities was monitored by employing a thermal imaging system (Fig. 9 and 10). Recent studies using thermal imaging systems have shown promising results in differentiating plant responses to soil moisture stress. For instance, under water stress conditions at 60% FC, 'Bhima Shakti' exhibited a cooler canopy temperature (36.5°C) compared to 'Bhima Shweta' (38°C) (Fig. 9 and 10). At moderate stress (60% FC), the canopy temperature of 'Bhima Shakti' was lower by 1.5°C as compared to 'Bhima Shweta' (Fig. 9 and 10). An increase of 1°C in canopy temperature is related to a 10% decrease in transpiration (Helyes et al., 2010). A cooler canopy was revealed in 'Bhima Shakti' by preponderance of a significantly higher number of cooler pixels in the thermal images of this genotype relative to 'Bhima Shweta' (Fig. 10). This difference in canopy temperature could be attributed to differences in stomatal regulation between the two genotypes. Overall, the results suggest that thermal imaging could be a useful tool for differentiating plant responses to soil moisture stress and for assessing the performance of different genotypes under these conditions.

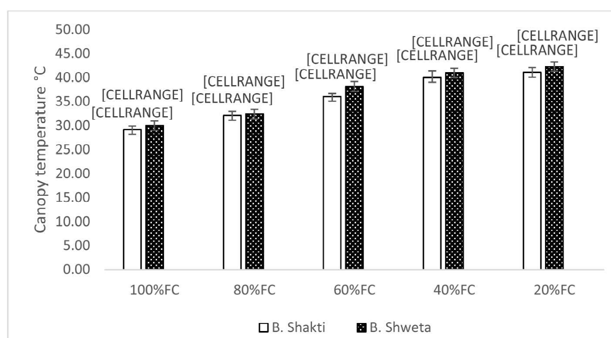
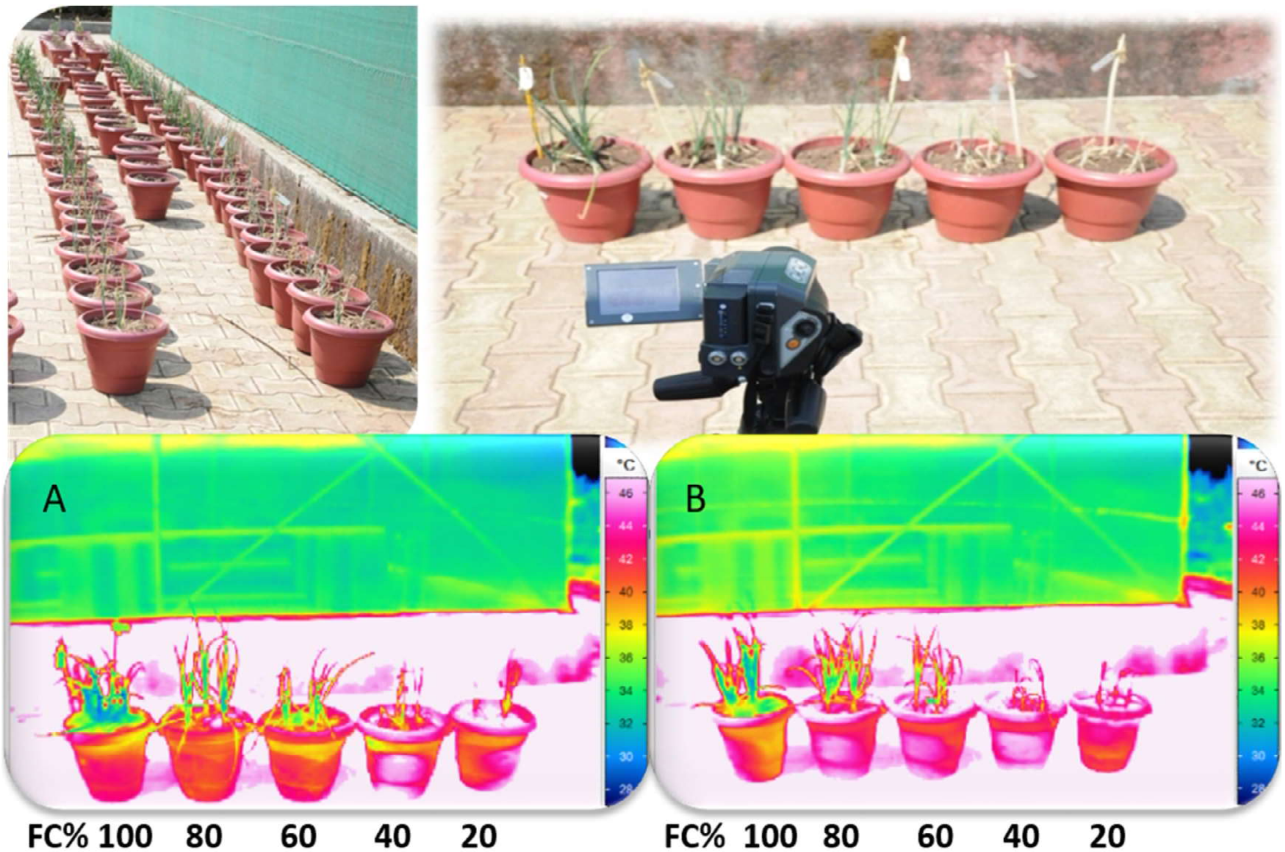


Fig. 9 : Effect of water stress on canopy temperature of onion genotypes



Bhima Shakti Bhima Shweta

Fig. 10 : Intensity of water stress on onion as indicated by visible and infrared images at different field capacities % (100, 80, 60, 40 20). (A) Bhima Shakti and (B) Bhima Shweta

CONCLUSION

This experiment demonstrated that water stress has a significant impact on the growth, physiological and biochemical characteristics of onion genotypes. The results showed that different water stress levels had a notable effect on all the parameters, with 'Bhima Shakti' demonstrating the most promising results. This genotype exhibited increased relative water content, lower lipid peroxidation, and lower canopy temperature. While all parameters did not provide clear indications of genotype superiority under stress conditions measuring relative water content, lipid peroxidation, and canopy temperature could be an effective way to phenotype onion genotypes against water stress.

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