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EFFECT OF HIGH-TEMPERATURE, DROUGHT, AND NUTRIENT AVAILABILITY ON MORPHO-PHYSIOLOGICAL AND MOLECULAR MECHANISMS OF RAPESEED - AN OVERVIEW

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Abstract

Production of high yielding cereals and oilseed crops is a major challenge for the farming community to meet the increasing global food demands. In many areas, global food production is challenged by abiotic and biotic factors which adversely hamper the growth of plants. Abiotic stresses, for instance, water, temperature, and essential soil nutrients stress affect the plant's growth. Plants have a different mechanism, which enables them to complete their life cycle under stress conditions, for example, some plants use environmental signals to regulate their morpho-physiological and molecular mechanisms. There is a huge gap of knowledge to identify the genetic mechanisms to mitigate the abiotic stresses. Given the current state of understanding; we have consciously focused on the effect of multiple abiotic stress-signals on morpho-physiological and molecular mechanisms of *Brassica napus*. Further, this review emphasizes the link between different abiotic stresses (high temperature, drought, and low nutrients availability) which ultimately regulate the growth and development of *Brassica napus*.

Key words: *Brassica napus* L.; Stress; Drought; Temperature; Nutrients.

Introduction

Plants are non-motile organisms that suffer from water, temperature, and nutrients stress during their growth and development (Kauser *et al.*, 2006; Ahmad & Prasad, 2011). Environmental conditions are changing very rapidly, which are creating difficulties for the survival of plants. Extreme environmental conditions (high temperature, drought, and low availability of nutrients) affect the morpho-physiological processes of plants ultimately affect the yield of crops. The agricultural sector is one of the key economic components of many agricultural countries in the world. Abiotic stress has the main impact on crop productivity worldwide, decreasing average yields for major crop plants (Kumar, 2013). Abiotic stress conditions enable the plants to develop defensive mechanisms to survive in harsh conditions, which affect the growth and development by affecting the physiological function of plants (Bray, 2000). These environmental stressful conditions affect the expression of particular genes that are responsive under stress conditions at the molecular level (García *et al*., 2020). Due to the non-motility plants respond to the outer stressful conditions by changing internal physiological functions, the gene expressive activity, and metabolic processes (Macedo, 2012), plants. Abiotic stresses are creating staggering challenges for biologists to solve the alarming conditions, which will be fatal for future cop production. Therefore, there is a dire need to investigate the effect of multiple abiotic stress conditions on morpho-physiological and molecular mechanisms of plants to cope with future environmental challenges (Macedo, 2012).

One of the major oilseed crops of the world is mustard which mainly includes *Brassica napus*, *Brassica juncea*, and *Brassica rapa*. Among these, *Brassica napus L* is mainly cultivated worldwide (Raman *et al.*, 2014). It has increasingly become an important agro-economic crop that is widely used for vegetative oil, animal feed, alternative fuel, etc., (Lee *et al.*, 2016; Wang *et al.*, 2018). Canola oil is reported as the third most important plant oil in the world after soybean and palm oil. Its seeds contain 40-50% oil (Chew, 2020). After the extraction of oil from the seed, it contains 38-44% protein which is used for animal nutrition in the form of seed cake. The production of *B. napus* is badly restricted due to the current situation of global climate change, frequent occurrence of extreme weather, water deficit, extreme temperatures, and other natural disasters (Li *et al.*, 2015). Drought is one of the major abiotic stresses, which is limiting crop growth, development and yield. Drought induces excessive ROS production, which damages cell membranes, plant DNA and proteins). Moreover, drought decreases the photosynthesis and biomass production of plants (Cechin *et al.*, 2007). It was studied that the cultivation of *Brassica napus* under drought conditions decreased the growth, grain yield and seed quality (Wan *et al.*, 2009). Therefore, there is an increased demand for the development of *B. napus* varieties that have a high yield, quality and resistance to adopt abiotic stresses (Wang *et al.*, 2018). Earlier researches provide pieces of evidence that seed yield can be increased by early planting (Degenhardt & Kondra, 1981; Angadi *et al.*, 2000). But it may be restricted due to spring frosts or excess moisture in fields (Koscielny *et al.*, 2018).

Therefore, there is a dire need to investigate the conditions in which yield and quality of *Brassica napus* are very sensitive to stress conditions so that the cultivation techniques or new varieties can be developed to maintain the productivity and quality of rapeseed crop. Plants also require nutrients for their normal physiological functions to increase productivity. The nutrients unavailability in the soil is the major determinant of *Brassica napus* production. Though some progress has been made to increase the production of *Brassica napus*, still there is a milestone to achieve in understanding how its productivity and oil quality is challenged by various environmental cues. Moreover, the morpho-physiological and molecular aspects of *Brassica napus* are still needed to be known in acclimation to numerous environmental conditions. In the present study, an insight into the various morpho-physiological and molecular aspects of *Brassica napus* with abiotic stresses has been given. Furthermore, we comprehensively elaborated the interlink between a variety of climatic factors that regulate the growth and development of *Brassica napus* and its adaptability to harsh environmental conditions.

Water availability: Water is a very important factor that influences crop morpho-physiological processes (Deng *et al.*, 2005; Ashraf *et al.*, 2007; Micheletto *et al.*, 2007). Water transportation between leaf and xylem is carried out due to water potential difference, but drought conditions reduce this potential difference, which disturbs the availability of water and nutrients for cell division and expansion. The low turgor pressure and slow photosynthetic rate under water scarcity reduce leaf size and ultimately limit yield due to low availability of assimilates. Water scarcity decreases germination potential, early seedling growth, root and shoot dry weight, hypocotyl length, and vegetative growth and ultimately decreases the yield of crops (Zeid & Shedeed, 2006). Moreover, water scarcity affects root morphology by decreasing the shoot-to-root mass ratio, increasing the growth of minute and thin roots, and decreasing the number of thick roots (Nosalewicz *et al.*, 2016). The effect of water scarcity depends upon the stress intervals, genotypes, stress severity, and growth phases of *Brassica napus*. Deficiency of water causes a reduction in cell division and expansion which retard plant height, leaf expansion, number of branches, oil content in seeds, chlorophyll content, and rate of photosynthetic activity of canola cultivars (Moaveni *et al.*, 2010). Drought conditions cause a reduction of yield by decreasing the siliquae number per plant, seed number per siliquae, 1000 seed weight, and oil content (Diepenbrock, 2000).

Among phytohormones, abscisic acid produces during water stress conditions and plays their important role in the stomata closure to cope with water scarcity (Hussain *et al.*, 2010). Closure of stomata decreases the availability of CO_2 for photosynthetically active cells. However, the reduction of CO_2 reduces the assimilates availability for vegetative and reproductive parts of the plants due to decline in photosynthesis, which ultimately reduces *Brassica napus* yield (Reddy *et al.*, 2004; Qaderi *et al.*, 2007; Yang *et al.*, 2009; Khan *et al.*, 2012), which is illustrated in (Fig. 1.) Abscisic acid (ABA) limits the production of ethylene and maintains plant growth (Yang & Hoffman, 1984; Srinivasarao *et al.*, 2003; Hussain *et al.*, 2010). The molecular studies on drought stress in plants have revealed that the *DREB* is the most important transcriptional factor as their expression increased by drought stress in the *Arabidopsis thaliana* (Kudo *et al.*, 2019). Another very recent investigation had introduced the new transcriptional factor i.e. *CLE25*. They proposed that *CLE25* was regulated by ABA (Christmann & Grill, 2018; McLachlan *et al.*, 2018). However, more information is required to find out the exact role of *CLE25* in water shortage conditions. Moreover, *Hsfs* and *WRKY* gene families are of great interest during stressful conditions. Among *Hsfs, the HsfA1a*, *HsfA1*, *HsfA2*, and *HsfA3* are the most important ones (SANMIYA *et al.*, 2020). More specifically, the expression of *HsfA3* was upregulated under stress in *Arabidopsis thaliana* (Yan *et al.*, 2020). Interestingly, *HsfA3* has a thermotolerance role during water scarcity and its expression is controlled by *DREB2A* transcriptional factor. *Hsf*A4 plays their interesting role in overcoming reactive oxygen species (ROS) to help the plant to withstand drought conditions (Zhu *et al.*, 2017). Generally, water stress decreases the grain yield, seed oil content (Alamisaied & Gharineh, 2008), and alters the lipids composition (Ullah *et al.*, 2012) and amino acids (glutamate & glutamine) quantity (Lohaus & Moellers, 2000; Norouzi *et al.*, 2008). Plants physiological processes, for example, leaf development, photosynthesis, and stomatal conductance are negatively correlated with water stress (Gammelvind *et al.*, 1996; Qaderi *et al.*, 2006; Müller *et al.*, 2010). It has been reported in *Brassica napus* that drought stress-induced the leaf wilting (Balestrini & Vartanian, 1983). Plant growth and development is highly influenced by drought as it decreases photosynthesis and osmolyte biosynthesis (Malinowski & Belesky, 2000), which change assimilates portioning (Andersen *et al.*, 1996). Plants close their stomata during drought to overcome transpiration losses and to maintain water potential. Closure of stomata decreases photosynthesis, which decreases yield (Gao *et al.*, 2018).

It was investigated by several researchers that water deficit conditions decreased plant height, branch number, siliqua per plant, seed per siliqua, 1000-seed weight, seed yield, biological yield, and oil yield (Masoud, 2007; Rad & Zandi, 2012; Naderi & Emam, 2014). A 2.4-fold increase in yield of cultivar Hyola 357 Magnum in response to 30 cm irrigation was recorded with an 8% increase in the oil content. Yield for rain-fed treatment (RF) was reported i.e. 1220 kg/ha in contrast to 2905 k/ha for maximum irrigation (HI i.e. 30 cm) (Pavlista *et al.*, 2016). (Rad & Zandi, 2012) reported that rapeseed cultivar (Hyola 401) produced a yield of 4588 kg ha^{-1} under normal irrigation while the cultivar RGS003 produced a maximum seed yield of 3577 kg ha^{-1}under water deficit conditions. Moreover, in another study, it was reported that 2138 kg/ha oil yield was obtained in control treatment while stress leads to a reduction of 877, 1141, and 1265 kg/ha after stem elongation, flowering, and pod formation stages in canola, respectively (Fard *et al.*, 2018). Increasing the moisture content after the pollination stage can increase seed and oil yield whereas water deficit stress at the end of the growing season reduces the percentage of seed oil (Masoud, 2007). The most important component of seed yield in rapeseed is the number of siliquae per plant is (Miller *et al.*, 2003). (Rad & Zandi, 2012) reported a 37% decrease in the number of siliquae per plant when exposed to drought stress whereas a maximum of 325 siliquae per plant was obtained from the normal irrigation. Another study on rapeseed cultivars has concluded that a 59% decrease in the number of siliquae per plant in response to drought stress was observed (Rad & Zandi, 2012). The main determinants of the quality of canola oil are the amount of oleic, linoleic, and erucic acids (Masoud, 2007). Previous studies have reported that the amount of saturated fatty acids of seed oil decreased in response to drought stress (Ahmadi and Bahrani, 2009; Shekari *et al.*, 2016). Some previous studies on the molecular regulation of drought stress in brassica plants have reported that among *WRKY* family *WRKY145, 142, BnaWRKY242, 141, 009,* and *BrWRKY51, 65, 98,* and *104* genes showed their expression during the drought in brassica plants (He *et al.*, 2016). Besides, many studies are conducted to exploring the molecular pathways in regulating the association of different environmental factors. But there is still a huge gap of information in elucidating the molecular regulations of brassica plants in response to drought stress.

How to cope with drought stress: Many strategies are adopted in crop production to enhance water conservation and water use efficiency (WUE). Planting methods were also used to cope with drought stress (Ijaz *et al.*, 2015). There are many important water conservation methods adopted by many farmers like drill sowing, raised bed planting, and furrow planting to ensure the availability of water to seeds during water scarcity (Zhang *et al.*, 2007; Kukal *et al.*, 2010; Aiken *et al.*, 2015). Among plant hormones, plant growth regulators are organic compounds that are also used to promote physiological processes in plants under drought conditions. Plant growth regulators promote, roots, stem, and leaves growth, seeds germination, flowering, and fruit ripening, etc. Exogenous applications of different organic compounds can mitigate the adverse effects of droughts on crop plants (Aown *et al.*, 2012). Application of plant growth regulators such as gibberellic acid, cytokinins and salicylic acid modify plant responses under drought conditions (Farooq *et al.*, 2009). The application of putrescine and salicylic on leaves significantly alleviate the harmful effects of water scarcity on rape (Ullah *et al.*, 2012). *Brassica napus* plants applied with putrescine and salicylic acid had higher relative water contents, carotene contents, chlorophyll contents, and proline contents under drought conditions as compared to untreated plants (Ullah *et al.*, 2012). During the flowering drought increases the concentration of glucosinolates, which produces pungent oil odors (Bouchereau *et al.*, 1996). Application of putrescine to rape plants under water scarcity conditions decreases the accumulation of glucosinolates concentration. Erucic acid produces pungent odors in rape oil, which can be reduced by the application of salicylic acid (Ullah *et al.*, 2012).

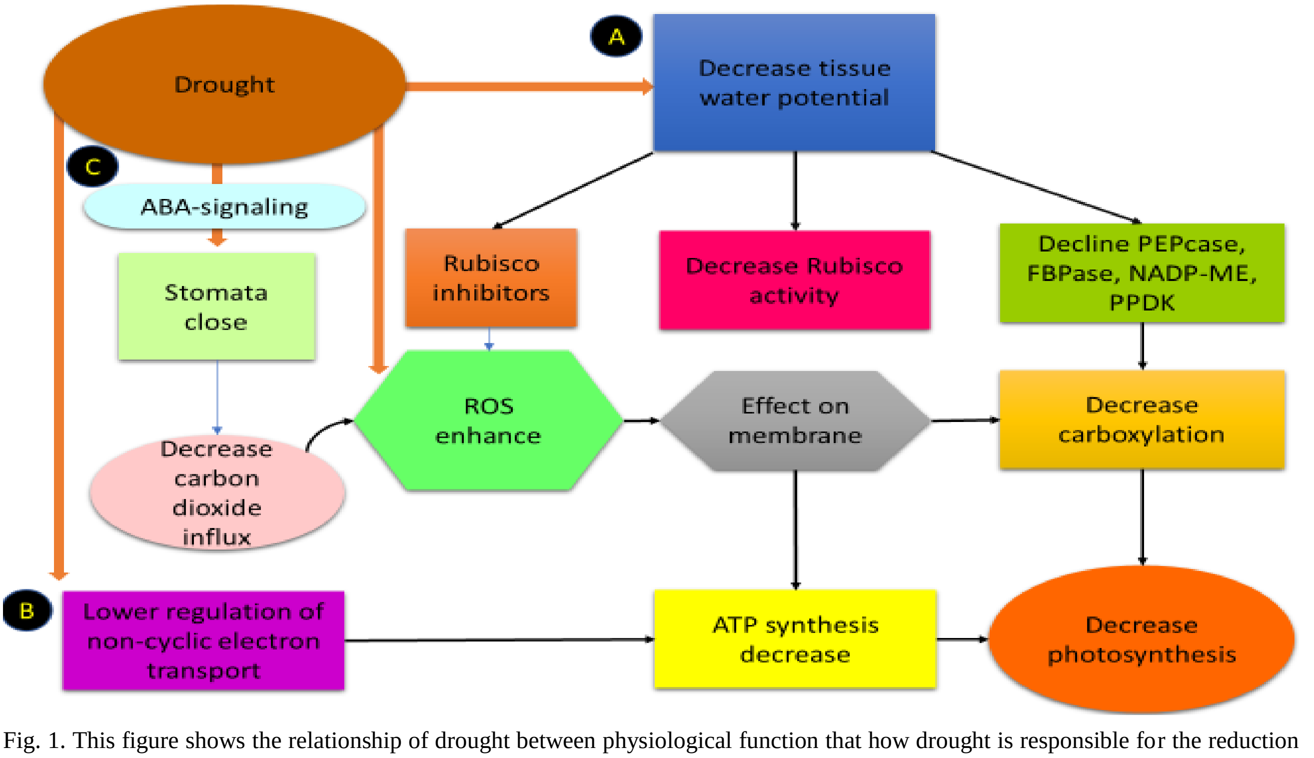

Fig. 1. This figure shows the relationship of drought between physiological function that how drought is responsible for the reduction in photosynthesis which ultimately causes a reduction in yield, (A) drought decrease water potential in tissues which reduce, Phosphoenolpyruvate carboxylase (PEPcase), Fructose 1,6-bisphosphatase (FBPase), Nicotinamide adenine dinucleotide phosphate-dependent malic enzyme (NADP-ME), Pyruvate phosphate dikinase (PPDK) and Rubisco enzymatic activities and (B) regulation of non-cyclic electron transport, (C) and triggers ABA signaling, plant respond to close their stomata to prevent water loss, close stomata don't allow entry of CO_2, plant increase reactive oxygen species (ROS). Drought decrease photochemical processes which cause the reduction of ATP synthesis, photosynthesis decline, ultimately cause in decrease crop yield.

Among all other nutrients, potassium acts as a primary osmoticum in maintaining low water potential in plant tissues. Potassium ions (K^+) accumulate in plant tissues under drought conditions to increase water uptake along with a soil-plant gradient (Glenn *et al.*, 1996). Another study strengthens this claim and it has been proved that the application of K fertilizer helps in mitigating the adverse effects of drought on plant growth (Andersen *et al.*, 1992; Sangakkara *et al.*, 2001). Moreover, potassium increases plant resistance against drought through its functions in stomatal regulation, osmoregulation, energy status, charge balance, protein synthesis, and homeostasis (Pandey, 2015).

Specifically, the application of potassium sulphate to *B. napus* under drought conditions helps in boosting seed yield, seed/siliqua and siliqua/plant (Ardestani & Rad, 2012). An increase in seed yield and dry matter of Indian mustard was reported by using higher amounts of potassium under drought stress. Potassium usage under water deficit conditions can prevent losses in seed yield of rapeseed. Increased use of potassium leads to a maximum number of siliquae per plant (Cheema *et al.*, 2012; Neseim *et al.*, 2014). An application of 200 kg/ha of K_2SO_4 in severe moisture conditions (i.e. irrigation after 80% soil water depletion) and normal moisture conditions can help to increase the yield of rapeseed by 59.95% and 21.48%, respectively (Ardestani & Rad, 2012). (Fanaei *et al.*, 2012) reported a maximum seed yield of 2975 kg/ha by the application of 250 kg/ha K_2SO_4, which was 21% more than the control ones (no potassium application). Furthermore, (Jianwei *et al.*, 2007) recorded a significant increase of 17.5 and 31.7% in grain yield of rapeseed by using 150 and 300 kg/ha K_2O, in comparison to control. Despite having these findings, there is still a big gap of information in elucidating the mechanisms of this interlink between drought and nutrients. More specifically, there is a big challenge for future biologists to find out the molecular signaling pathways in controlling the association between drought and nutrients uptake in brassica.

Effect of high temperature: The international report on the Climate in 2017 states that the earth surface temperature has increased 0.7–0.9° Celsius (1.3–1.6° Fahrenheit) in the hundred years since 1901, but from 1975 temperature increased almost in double 1.5–1.8° Celsius (2.7–3.2° Fahrenheit). Temperature affects plant growth and development by various means. High-temperature stress causes a decrease in oil content, and an increase in protein content and saturated fatty acids. The enzyme activity and respiration rates are increased by a rise in temperature (Aksouh-Harradj *et al.*, 2006; Arcus *et al.*, 2016). High leaf temperature increases the photorespiration rate, which decreases the photosynthetic activity (Long, 1991). Furthermore, an increase in temperature decreases the specificity of rubisco for CO_2 vs O_2 hence the oxygenation reaction will occur (Ku & Edwards, 1978; Jordan & Ogren, 1984). The solubility of CO_2 rises than O_2 by increasing temperature (Ku & Edwards, 1977). The activation state of rubisco decreases as the leaf temperature rises (Crafts-Brandner & Salvucci, 2000; Yamori & von Caemmerer, 2009). At high temperatures, the production of ATP, NADPH, and photosynthetic electron transport rates decreases, which ultimately decreases photosynthesis (Schrader *et al.*, 2004; Wise *et al.*, 2004). Hence yield of crops decreases by decreasing the photosynthetic rate. The effect of temperature stress on crop plants is illustrated in (Fig. 2). Specifically, the previous study on *Brassica napus* observed the effect of heat stress by using hybrids and inbred with two different planting dates and found that heat stress caused a 20% reduction in seed yield of hybrids and 25% reduction within the inbred (Koscielny *et al.*, 2018). This indicated that heterosis can reduce the effect of high-temperature stress on the yield of canola. (Grant & Beversdorf, 1985) reported an increase in seed yield and decrease in protein content, glucosinolate levels under optimal conditions due to heterosis.

Plant growth and development are affected due to an increase in temperature by global warming, certainly at the early stages of plant growth, which is an emerging constrain in several cultivated areas of the world. High temperatures cause a delay in the normal morpho-physiological processes of several plant species and increase the probabilities of yield losses. 28°C is the optimum temperature for germination of *Brassica napus* and any increase in temperature above this level delays growth and development. Tropical areas have high temperatures, which cause scorching of leaves and twigs, leaves senescence, and discoloration of fruits and leaves (Vollenweider & Günthardt-Goerg, 2005). High temperatures cause an increase in proline and a decrease in chlorophyll content (Gupta *et al.*, 2013; Shah *et al.*, 2015). An increase in temperature above optimum temperature leads to floral sterility and reduces flower number which causes a reduction in yield of economically important *Brassica napus* species, while the high temperature at the flowering and grain filling stage affects pollen variability, seed development, anthesis, and fertilization process.

Plants respond to heat stress at the transcriptional level by showing the functions of heat stress transcriptional factors (*HSFs*). *HSFA1* is a very important protein that regulates the other *HSFs* (LIU *et al.*, 2011). Interestingly, *HsfA3* has a thermotolerance role during water scarcity and its expression is controlled by *DREB2A* transcriptional factor. It means that these two proteins have some connections while contributing to the plant's physiology in response to environmental stimuli (Zhu *et al.*, 2017). Moreover, *HSP101* belongs to *HSPs* and is very helpful in thermotolerance during temperature stress (Su & Li, 2008). There are many gene families in Arabidopsis which play their role in abiotic stress conditions. *PIF4* is a gene that belongs to the *PIF* gene family, shows its expression under high-temperature stress and the *TCP5* transcriptional factor holds over the plant thermogenesis by controlling the *PIF4* activity (Han *et al.*, 2019). Down-Regulation of the *BnWRI1* gene occurs during temperature stress conditions. *BnWRI1* showed overexpression under temperature stress to maintains the process of photosynthesis and oil accumulation in rapeseed. Although a lot of studies have been done on the morpho-physiological responses of *Brassica napus* in the regulation of stress signal perception, however, there is a dire need to elucidate the molecular mechanisms in mediating the association between different abiotic stresses. More specifically, how does *HsfA3* has crosstalk with *DREB2A* in mediating the plant responses under drought and heat stress?

Effect of nutrients: Plants require nutrients for carrying out their normal physiological functions. Major nutrients are NPK, which are very important for plants (Khan *et al.*, 2005). There are some nutrients, which play a very important role in the yield of *Brassica napus*, and these are given below.

Nitrogen is a very vital part of a plant as it is a structural component of chlorophyll. It was investigated that high nitrogen application rates increased the glucosinolate content, but oil content decreased (Rathke *et al.*, 2005; Cheema *et al.*, 2010). There should be the use of a recommended dose of N because it increases the economic yield and minimizes environmental pollution (Aufhammer *et al.*, 1994; Mason & Brennan, 1998; Khan *et al.*, 2005). Higher nitrogen rate increased lodging, decreased yield, changed fatty acid composition, enhanced palmitic and stearic acid content, but linoleic and linolenic acid content decreased, hence poorer oil quality by increasing erucic acid and glucosinolate content (Khan *et al.*, 2018).

After nitrogen, phosphorous is the most important nutrient for plants because it is not easily available for plants due to its low solubility in soil (Raghothama & Karthikeyan, 2005). Phosphorous is a very important and essential element of oil because it is a component of proteins that determining its essentiality for *Brassica napus* (Cheema *et al.*, 2010). Due to the low availability of phosphorous, there is a need to develop suitable conditions that would help *Brassica napus* plants for carrying out their normal morpho-physiological functions. *Brassica napus* require high availability of phosphorous for optimum yield. Low availability of phosphorous decreases the yield of *Brassica napus* (Zhang *et al.*, 2009). Phosphorous is present in excess amounts in the upper layer of soil but its availability is not in access for plants due to its unavailable form. Phosphorous deficiency increases the activity of acid Phosphatase (APase) in the soil (Zhu & Smith, 2001). Nutrients deficiency not only affects the plant physiology but also plays a role at a molecular level in mediating the expression of genes like *UBC21* and *HTB1* which are very responsive in phosphorous deficiency. *BnTrx1;1* and *BnPht1;3* show their expression under nutrient deficiency. *BnPht1;3* shows their expression especially in roots under phosphate deficiency, however, *BnTrx1;1* gene expresses itself under high phosphorous deficiency especially in roots and leaves (Wang *et al.*, 2018). A very recent study has reported that *BnaNACED3* is a very important gene in *B. napus* which shows their expression by the regulation of ABA biosynthesis for growth, development, and stress adaptation (Xu and Cai, 2017). Although this study elucidated the role of *BnaNACED3*, however, there is still a big gap of information for future scientists to study its effect under the combined effect of nutrient and drought stress.

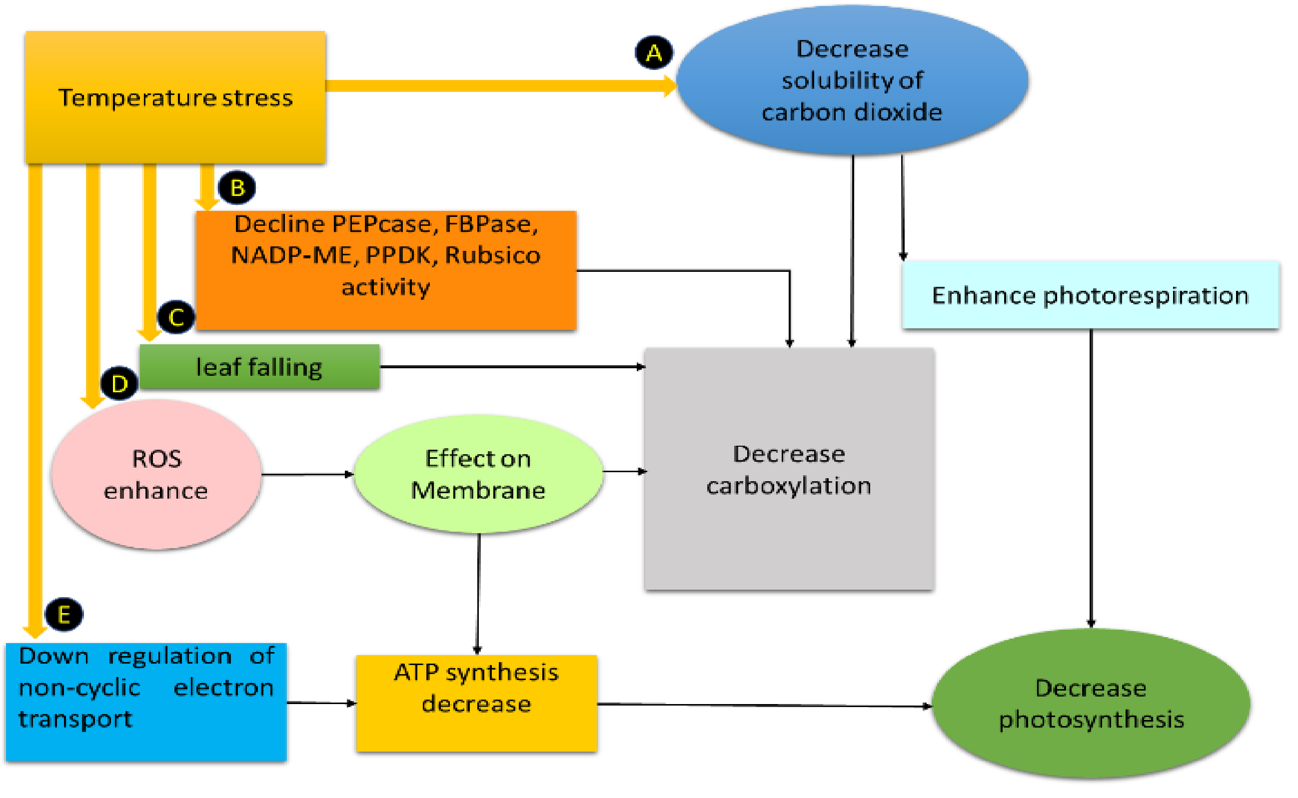

Fig. 2. This figure shows how temperature stress effective and the response of plants by changing their physiological functions, (A) temperature stress inhibits rubisco activity and reduces CO_2 solubility which decreases carboxylation and enhances photorespiration. (B) decrease, Phosphoenolpyruvate carboxylase (PEPcase), Fructose 1,6-bisphosphatase (FBPase), Nicotinamide adenine dinucleotide phosphate-dependent malic enzyme (NADP-ME), Pyruvate phosphate dikinase (PPDK), and Rubisco enzymatic activities. (C) and increase leaf senescence. (D) reactive oxygen species (ROS) production is a good indication of stress situations, which attack the cell membrane and relate to decreasing the process of ATP synthesis and decarboxylation. (E) Downregulation of non-cyclic electron transport causes a reduction of ATP synthesis which ultimately reduces the photosynthetic activity of crop plants.

Magnesium (Mg), another important nutrient for plant growth (Karley & White, 2009). Mg is a central structural atom of chlorophyll molecule which takes part in enzyme activities and chelation to nucleotidyl phosphate forms (Shaul, 2002). Its deficiency causes an increase in antioxidative mechanisms and increases the sugar levels, which is not effective for photosynthesis (Hermans *et al.*, 2005; Hermans *et al.*, 2010). The growth of young leaves decreased by the deficiency of Mg because it disturbs the distribution of carbon towards leaves than that to roots hence the reduction of root growth is less affected than leaves (Hermans & Verbruggen, 2005; Hermans *et al.*, 2006). Mg deficiency causes more Regulation of genes in leaves than in roots because it usually deals with chlorophyll and its related components (Hermans *et al.*, 2010). Though it is a very well-known nutrient to regulate plant growth and development. Plenty of studies have been done to demonstrate Mg role in plants, however, it is one of the most interesting topics to explain its functions in *Brassica napus* and how it is involved in making the connection between different abiotic stresses. (Kashem & Kawai, 2007), reported that application of Mg to *Brassica napus* plants growing under hydroponic conditions suffering from Cd toxicity nutrients solution increased the growth and development by detoxifying the Cd toxicity in plants especially shoot.

Another nutrient, cadmium (Cd) is absorbed by roots through plasma membrane-like as iron (Fe), calcium (Ca), and zinc (Zn) (Clemens, 2006; Hermans *et al.*, 2010; Lux *et al.*, 2010). Cd reacts with sulphydryl groups and becomes a source of toxicity. Cd can replace the position of Mg in chlorophyll structure which damage the photosynthetic structure by stopping the function of photosystem Ⅱ (Faller *et al.*, 2005) or by disturbing the biosynthesis of chlorophyll (Gadallah, 1994), arran**g**ement and construction of light-harvesting complexes (Janik *et al.*, 2010) and chloroplast organization and thickness (Baryla *et al.*, 2001; Carrier *et al.*, 2003). However, this phenomenon still needs to be studied at the molecular level that how do Cd affects the efficiency of chlorophyll? And what are the key genes that are involved in the molecular regulation of the chlorophyll? Higher Cd availability in the growth medium can stop the uptake of Fe in many species (Kovács *et al.*, 2006). Deficiency of Fe occurred due to the Cd introduction, which leads to the development of chlorosis (Kramer *et al.*, 1991; Su & Li, 2008). Cd is one of the most important heavy metals and its increasing toxicity in the world is hazardous for human health. The stress of Cd is harmful to normal morpho-physiological functions in plants especially for brassica crop plants because its stress reduces growth, development, and oil content (Ahmad *et al.*, 2015). *Brassica* spp. is very famous in the world as a phytoremediator because it can accumulate heavy metals (Mourato *et al.*, 2015).

Drought and nutrients uptake: Nutrient uptake is one of the important processes for the proper growth and development of plants. Plants usually uptake nutrients under the presence of water, water scarcity can affect the nutrients uptake. Water scarcity greatly affects the uptake of essential nutrients. Roots uptake many nutrients under the presence of adequate water like nitrogen, silicon, magnesium, and calcium, drought limits the movement of these nutrients by affecting the process of diffusion and mass flow and ultimately retard the plant growth (Barber, 1995). Drought conditions change the root morphology, for instance, root length and surface area, these alterations in root architecture are helpful to plants for capturing low mobile nutrients (Lynch & Brown, 2001). The deficiency of water retards the growth of plant roots which is a hurdle to plants to uptake low mobile nutrients like phosphorous (Garg, 2003). Microbial activities and their interaction with plant roots play a very important role in nutrients uptake from soil to plants. Drought conditions negatively affect the microbial activities hence reduce the nutrient supplying ability of microbes for plants (Schimel *et al.*, 2007). Different crop species behave differently under water scarcity conditions. Usually, N uptake is enhanced, P uptake is decreased and K uptake remained unaffected under water deficit conditions. Although the relationship among the nutrients is very complicated due to the interactive effect on each other and overall plant physiology. This particular aspect requires more research at a sophisticated molecular level.

Future perspectives: Agriculture is one of the most important economic sectors, it is the economic backbone of all the world and its sustainability depends upon the potent nutrients availability and suitable environmental conditions. The evolution of plants has been influenced by various harsh environmental conditions, which include a shortage of water, extremes of temperature, unavailability of nutrients in the soil profile. As a result of these severe environmental conditions plants have acquired mechanisms to sustain stability among growth, development, and reproduction.

There is a dire need to discuss conditions in which the yield and quality of *Brassica napus* are very sensitive to external factors especially, under stress conditions so that the productivity and quality of this crop could be maintained. Moreover, plants require nutrients for carrying out their normal physiological functions. The nutrients availability in soil is the major determinant of crop productivity of *Brassica napus*. Though many signs of progress have been done to increase the production of *Brassica napus*, still there is a milestone to achieve in understanding how its productivity and oil quality is challenged by various environmental cues.

The morpho-physiological and molecular aspects of *Brassica napus* are still needed to be known in acclimation to numerous hazardous environmental conditions. In the present study, an insight into the various morpho-physiological and molecular aspects of *Brassica napus* with abiotic stresses has been given as illustrated in (Fig. 3) which depicts the questions for future scientists to investigate the multiple abiotic stress factors. However, there is a huge gap of information in elucidating the molecular regulations of *Brassica napus* in response to drought stress especially the newly founded gene *CLE25* (Fig. 3C). How this gene does regulate the molecular regulations of *Brassica napus*? More specifically, how does *HsfA3* has crosstalk with *DREB2A* in mediating the plant responses under drought and heat stress (Fig. 3A)? In context to nutrient availability and their interaction with other abiotic stresses, there would be plenty of questions to be asked for future scientists. For instance, how does Cd affect the efficiency of chlorophyll, when it acts with magnesium in *Brassica napus* (Fig. 3B)? What are the key regulators of this action at the molecular level? It is discussed that potassium could be helpful to mitigate drought, whereas there is a question arises that how does the potassium interlink with ABA to regulate the phenomenal change in *Brassica napus*? What are the inducible genes and transcriptional factors that are involved in this regulation? Broadly, it one of the most fascinating topics for future studies to investigate the combined effect of abiotic stresses, and to find out the link between multiple environmental stresses in *Brassica napus*.

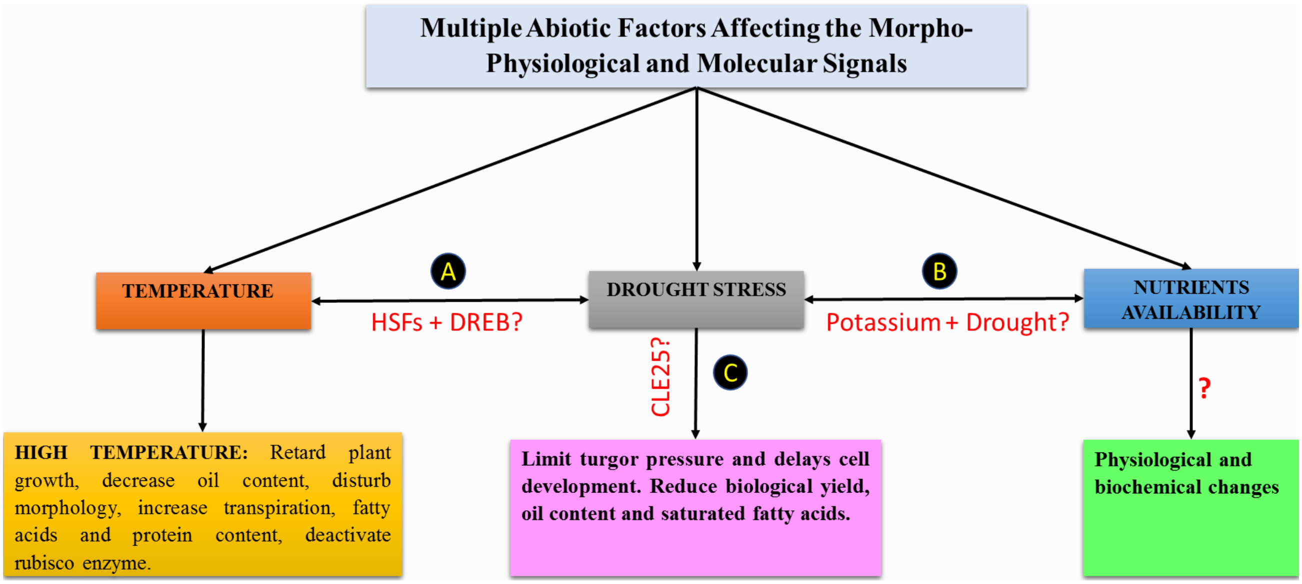

Fig. 3. This figure shows the relationship of multiple factors on Morpho-Physiological processes of plants, (A) shows how temperature and drought coordinate with each other in terms of HSFs and DREB interaction. (B) what is the key role of Potassium (K) under drought conditions, the role of nutrients on physio-chemical changes in plants? (C) This shows that, how does CLE25 activate under drought stress, and what is the exact role of this gene?

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