

**Enhancing physiological efficiency and yield potential
of Indian mustard using hydrogel and salicylic
acid under rainfed condition**

**By
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(2018BS16D)**

*Thesis submitted to Chaudhary Charan Singh Haryana
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**DOCTOR OF PHILOSOPHY
IN
PLANT PHYSIOLOGY**



**COLLEGE OF BASIC SCIENCE AND HUMANITIES
CCS HARYANA AGRICULTURAL UNIVERSITY
HISAR-125004, HARYANA, INDIA**

2023

CERTIFICATE-I

This is to certify that this thesis entitled “**Enhancing physiological efficiency and yield potential of Indian mustard using hydrogel and salicylic acid under rainfed condition**” submitted for the degree of **Doctor of Philosophy** in the subject of **Plant Physiology** to the **Chaudhary Charan Singh Haryana Agricultural University, Hisar**, is a bonafide research work carried out by **Ms. Neelam Rani, Admn. No.2018BS16D** under my supervision and guidance and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigations have been duly acknowledged.

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This is to certify that this thesis entitled “**Enhancing physiological efficiency and yield potential of Indian mustard using hydrogel and salicylic acid under rainfed condition**”, submitted by **Ms. Neelam Rani, Admn. No. 2018BS16D** to the **Chaudhary Charan Singh Haryana Agricultural University, Hisar** in partial fulfillment of the requirement for the degree of **Doctor of Philosophy** in the subject of **Plant Physiology**, has been approved by the Student’s Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.

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(Neelam Rani)

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ABBREVIATIONS AND SYMBOLS

AA	Ascorbic acid
APS	Ammonium per sulphate
APX	Ascorbate peroxidase
DHA	Oxidized ascorbate
AsA	Reduced ascorbate
ATP	Adenosine tri phosphate
CAT	Catalase
CD	Critical difference
DAS	Days after sowing
DMSO	Dimethyl sulphoxide
DW	Dry weight
dH ₂ O	Distilled Water
EDTA	Ethylene diamine tetra acetic acid
FW	Fresh weight
GK	Gond katira
GR	Glutathione reductase
HG	Hydrogel
MPa	Mega Pascal
MW	Molecular weight
NBT	Nitro blue tetrazolium
POX	Peroxidase
PSI	Photosystem I
PSII	Photosystem II
PVP	Polyvinyl pyrolidone
RBD	Randomized block design
ROS	Reactive oxygen species
RWC	Relative water content
SA	Salicylic acid
SOD	Superoxide dismutase
TTC	2, 3, 5–triphenyl tetrazolium chloride
Ψ_s	Osmotic potential
Ψ_w	Water potential

CHAPTER-I

INTRODUCTION

India is currently the most populous nation globally, with a notable increase in population growth rate. India's edible oil industry, After China, the United States, and Brazil, ranks as the fourth-largest in the world, producing 10% of the global oilseed production (James and Goli, 2016; Samir *et al.*, 2018). Moreover, oilseeds are cultivated on 14.8% of the nation's total cultivable land, making it the second-largest crop after cereals (Sharma, 2017a). India cultivates nine different oilseed crops each year under varying agroclimatic conditions, with rapeseed-mustard being one of the major producers, accounting for 25% of the nation's total oilseed production (Yadav *et al.*, 2019). Although India ranks seventh globally in terms of rapeseed-mustard production, it ranks second globally in terms of area and production. Despite this, India still imports about 57% of the world's edible oil, costing a staggering \$100 million US dollars annually in foreign currency (Jat *et al.*, 2019). Rapeseed-mustard is cultivated on 5.98 million hectares of land in India, producing a total of 8.4 million tonnes and boasting a productivity of 1410 kg/hectare (Anonymous, 2018). In terms of cultivation area and output, soybean is the only crop that surpasses rapeseed-mustard (comprising approximately one-fourth of the total oilseed area and production). However, rapeseed-mustard is the foremost producer of edible oil (Jat *et al.*, 2019). In addition, rapeseed-mustard accounts for 26.6% of India's total oil meal production and almost 20% of its total oil meal exports, cementing its position as the top oil meal exporter in the world (Sharma, 2017b). Indian mustard is the most extensively grown species of rapeseed-mustard in India, comprising roughly 90% of the nation's total rapeseed-mustard farming area. When compared to other crops, its oil contains a higher concentration of omega-3 fatty acids, is cholesterol and trans-fat-free, and has a lower N-6 to N-3 ratio (Jat *et al.*, 2019). The defatted cake produced by Indian mustard boasts a high protein content and a balance of essential amino acids, making it a superior source of nutrition for livestock and poultry (Sahni *et al.*, 2007). Despite India's vast territory and significant production of Indian mustard, our country's productivity is comparatively low when compared to other nations that grow mustard, including China, Canada, and the European Union, as well as the global average productivity (Kumar *et al.*, 2016; Yadav *et al.*, 2019). To meet the demand for edible oils for the projected 1.68 billion people in India by the middle of the twenty-first century, there must be a roughly three-fold increase in the production and a two-fold increase in productivity of rapeseed-mustard crops, along with a substantial increase in the production of other primary oilseed crops (Yadav *et al.*, 2019). To achieve the desired progress in the production of edible oils, it is imperative to

enhance both productivity and yield stability. India's oilseed production is on the brink of a Yellow Revolution in agriculture. The Solvent Extractors Association of India (SEA) launched the "Mustard Mission 2025" in 2021 to elevate mustard production to 20 million tonnes by that time. The implementation of improved farming techniques, utilization of appropriate technologies, cultivation of premium quality seeds, and the efficient management of inputs can facilitate the accomplishment of this goal.

The impact of drought as abiotic stress on plant growth and yield is significant on a global scale, as noted by Ma *et al.* (2020). Approximately one-third of the world's landmass and cropland are damaged by this phenomenon. Several states in India, including Rajasthan, Punjab, Haryana, Karnataka, Gujarat, and Maharashtra, are known to have insufficient rainfall. Rainfed agriculture refers to a type of farming that entirely depends on rainfall. India boasts 94 million acres of fertile land, 60 percent of which is rain-fed, and is home to 300 million people (Gupta *et al.*, 2011). This often leads to drought due to the infrequent, intermittent, and fleeting nature of rainfall in rain-fed drylands. A dry land is defined as a region where the estimated annual average rainfall required to cultivate crops is less than 500 mm. Unfortunately, this range of rainfall is insufficient for crop growth, as the rate of evapotranspiration is higher than that of moisture absorption (Gautam *et al.*, 2014). However, some states have implemented prophylactic irrigation, resulting in varying levels of impact during droughts. The well-irrigated regions, East UP, West UP, Haryana, Chandigarh, Delhi, and Punjab, are classified under the category of 4 meteorological sub-divisions (MSDs), with irrigation covering over 75% of the Gross Cropped Area (GCA). Despite being located in areas with low rainfall, these Sub-Divisions have a high prevalence of irrigation, with a normal rainfall level of 672mm, as noted by Jain (1972). In the country, around 44% of food is produced on roughly 56% of the cultivated land, which is dependent on rain. This makes the agriculture industry and food security of the country heavily reliant on monsoon rainfall. As a result, other economic sectors are also negatively impacted. Depending on the sowing time and winter rainfall, the crops are subjected to water shortage at one or more phenological stages. The shortage of water is mainly due to unpredictable rainfall and inadequate irrigation infrastructure caused by the soil's decreasing moisture levels. Brassica species are mostly grown in semiarid and arid climates. Drought stress results in disrupting both the osmotic and cellular ionic balance, as stated by Zhu and Gong in 2014 and the growth of plants is typically restricted by the availability of water. Both low water stress and excess water can negatively affect plant development and productivity, as evidenced by studies such as Riaz *et al.* (2010). In Brassica species, drought conditions have been found to significantly reduce output and seed yields. Plants have evolved various adaptations to survive in water-limited environments, including decreased leaf area, stomata closure to reduce transpiration, reduced stomatal conductance, limited internal CO₂ concentration, and decreased photosynthesis. Chaves *et al.*

(2003) and Karim *et al.* (2003) have documented these adaptations in detail. To increase plant productivity, humans can intervene by developing drought management techniques that reduce plants' sensitivity to water stress. As droughts with varying and unpredictable intensities affect mustard and other crops, according to Singh *et al.* (2017), the cultivation of crops requires immediate attention to drought-resistant methods. The main approaches to managing drought are to optimize the usage of present water resources and to reduce the negative effect of water scarcity on crop growth (Mehta, 2004). Hydrogel application, whether synthetic or herbal and salicylic acid has been proven to mitigate the catastrophic impact of drought and enhance crop yields.

Hydrogels, which are polymers that are cross-linked, have the potential to absorb water up to 1500 times their dry weight due to their interconnected network structure (Singh *et al.*, 2018). Significantly, when the temperature increases up to 50⁰C, the swelling rate of the hydrogel increases without disrupting the structure of the polymer matrix. Hydrogel is the term used to describe these synthetic materials, which come in crystal form and are marketed under various brand names such as Pusa Hydrogel and Super Absorbent. A recently developed semi-synthetic polymer known as "Pusa Hydrogel" has been created by the Indian Agricultural Research Institute (IARI). This innovative substance has the potential to increase the yield per unit of input. Furthermore, the application of Pusa hydrogel has been shown to improve the hydro-physical properties of soil, including porosity, aggregate stability, and hydraulic conductivity, as stated by Dabhi *et al.* (2013). Irrigation needs can be decreased across various crops such as cereals, pulses, vegetables, and flowers with the use of hydrogel, resulting in reduced water and labour expenses as well as savings in time and money spent on irrigation.

Although synthetic hydrogels like Pusa Hydrogels were initially intended for agricultural purposes in the 1980s, their high cost (Rs 1500–2,000/kg) has prevented them from becoming popular among farmers. The use of herbal hydrogel technology has proven to be highly effective in reducing irrigation water usage, resulting in increased plant growth and lower costs of production. By reducing the need for fertilizers, chemicals, water, electricity, and labour, this technology has also shown great potential in terms of generating high seed yields and environmental benefits. In particular, it has successfully allowed for the growth of rice crops in irrigated upland ecology and sandy soils in semi-arid northern India, and could potentially be utilized in other areas of the world with similar ecosystems (Lather, 2019).

Salicylic acid is a phenolic compound and has been established as a prominent growth regulator which has a significant impact on various biological traits of crops. It promotes several plant activities such as seed germination (Koo *et al.*, 2020), cation absorption and transport, photosynthetic processes, and growth rate and assists in mediating plant reactions to environmental stressors such as salinity and drought (Asif *et al.*, 2022).A

possible explanation for the effect of salicylic acid on plant abiotic stress responses may be its role in the development of stress-related symptoms. But it's also crucial in the adaptation process and in stimulating stress tolerance (Horvath *et al.*, 2007). SA instigates changes in rubisco levels, chlorophyll levels and protein kinase activity, while also promoting the production of heat shock proteins, chaperones, and dehydrin-like proteins. The expression of these genes may decrease ROS production in tissues that are actively participating in photosynthesis (Aldesuquy *et al.*, 2018). Various research has revealed that the application of salicylic acid has a positive impact on plants by shielding them from drought-induced oxidative damage. It has been suggested that the addition of salicylic acid to mustard plants leads to a rise in the amount of glucosinolates present in the leaves. When plant tissues are damaged, glucosinolates break down and discharge various compounds that are believed to assist the plant's protection against pests and bacteria. Ali (2021) found that the level of the secondary metabolite increased in plants in response to salicylic acid. Additionally, Yavas and Unay (2016) discovered that the introduction of exogenous salicylic acid into wheat plants led to a rise in proline and abscisic acid (ABA) levels. This increased the plant's resistance to salt stress.

According to a study conducted by Farhangi-Abriz and Ghassemi-Golezani in 2018, the use of salicylic acid treatment showed a notable increase in both soybean plant biomass and grain yield under salt stress. This was achieved by elevating levels of Ca^+ and K^+ ions, as well as chlorophyll content and CSI, while simultaneously reducing ionic, oxidative, and osmotic stresses. The activation of specific enzymes, including aldolase, reductase, and ascorbate peroxidase, was observed when tomato plants were cultivated from seeds that had been steeped in salicylic acid. The application of salicylic acid externally was found to increase the photosynthetic rate and membrane stability, resulting in the improved growth of barley plants under stress, as reported by EI Tayeb (2005).

This study aims to assess the effectiveness of hydrogel and foliar sprays of salicylic acid in reducing drought stress on mustard plants, providing a cost-efficient solution for addressing drought stress in rainfed conditions. The objectives of the study are as follows:

Objectives of Investigation:

1. To evaluate physiological characterization of Indian mustard under rainfed condition
2. To study the efficacy of hydrogel and salicylic acid for enhancing yield potential of Indian mustard under rainfed condition

CHAPTER-II

REVIEW OF LITERATURE

Drought is a prolonged period of months or years where a region experiences a scarcity of water, both above and below ground, caused by a consistent lack of precipitation. This global phenomenon has a random occurrence and can cause significant damage. Drought stress, a result of water scarcity, has a high influence on the yield of rain-fed crops, as noted by Kumar and Upadhyay in 2019. Deviations in rainfall patterns can also cause water stress, which affects all characteristics of plant growth and development, including cell division and expansion, leaf size and area, root-to-shoot ratio, number of nodes and branches, plant height, seeds/pod, and ultimately, yield. Alghabari *et al.* (2016) and Langadi *et al.* (2021) both have documented this phenomenon. As water scarcity becomes more prevalent, plants undergo a multitude of changes on a morphophysiological, anatomical, and molecular level. Under drought stress, morphological effects such as inhibited germination of seed and seedling growth are common (Harris *et al.*, 2007). Additionally, plant traits such as partitioning, harvest index, biomass accumulation, and crop productivity are all negatively impacted by drought stress. Morphophysiological disorders caused by drought stress also affect nutrient uptake and decrease the active movement of photosynthates, as well as alter factors such as RWC, leaf temperature and osmotic potential (Fanaei *et al.*, 2012).

The rapeseed mustard group of crops in India is grown across a broad agro-climatic range, spanning from the northwestern or northeastern hills to the rain-fed or under-irrigated regions in the south. These crops are planted either in a timely or late manner, in saline soils and mixed cropping systems. Brassica species are predominantly grown in the northern and eastern regions of the country. These species are typically cultivated in areas with high precipitation and tend not to perform well in regions with low rainfall (Richards, 1978). Drought conditions have significantly reduced the growth and production of seed yield for Brassica species. This is a major concern as drought stress is a primary factor in declining agricultural productivity, closely linked with other abiotic stresses such as heat stress and salinity (Mahmood *et al.*, 2009). The reduction in seed yield is particularly pronounced when water stress occurs during or after the flowering stage. This is likely due to the plant's susceptibility to anthesis, pollen development and fertilization, resulting in lower grain yield (Masud and Sinkai, 2007; Faraji *et al.*, 2009; Farooq *et al.*, 2009). Indian mustard is currently experiencing severe drought conditions with unpredictable and fluctuating intensities, resulting in a notable reduction in seed yield production. To combat this issue, it is suggested that a foliar spray of mineral elements, such as salicylic acid, and the application of hydrogels

(both synthetic and herbal) to the soil may help to alleviate the negative effects of water stress on yield reduction. Furthermore, studying the morpho-physiological and biochemical traits of mustard crops could enhance our understanding of how these nutrients affect the physiological mechanisms that allow plants to adapt and maintain growth and production during periods of water deficit. By doing so, effective drought management strategies can be devised. Thus, this investigation aims to determine the optimal dose of salicylic acid and hydrogels necessary to mitigate the adverse effects of drought stress in Indian mustard.

The topic of this chapter pertains to the research conducted on mustard concerning drought, which is a multifaceted subject. Despite the existing research on this topic, there is a dearth of information on the effects of drought on mustard concerning salicylic acid and hydrogel. In light of this, research findings on wheat, barley, gram, taramira, and other abiotic stresses have been integrated. This chapter provides a comprehensive review of relevant research conducted both in India and abroad, organized and presented in the following categories:

2.1 Effect of drought on growth and reproductive parameters

The attainment of growth is accomplished through a multitude of processes, including cell division, cell expansion, and bud differentiation. These processes are brought about by a complex interplay of genetic, physiological, ecological, and morphological events. The occurrence of these events is contingent on the quality and quantity of plant production and can be negatively impacted by water shortages. One of the physiological processes most vulnerable to drought is cell growth, due to the reduction of turgor pressure. In fact, under conditions of extreme water scarcity, cell elongation in higher plants can be inhibited by disrupting the flow of water from the xylem to surrounding cells, as reported by Farooq *et al.* (2009) research.

According to studies conducted by Gales and Wilson (1979) on winter wheat, Bannayan *et al.* (2008) on isabgol and black cumin, and Tabrizi (2004) on isabgol, drought stress was found to induce maturity stages in these species. However, the extent of induction was found to be dependent on several factors such as stress level, duration, species, and maturity stage. When a wheat plant is under drought stress, it employs a mechanism known as the induced maturity stage to avoid dehydration (Gales and Wilson, 1979). In separate studies, Razmjoo *et al.* (2008) and Baghalian *et al.* (2011) discovered that the height of *Matricaria chamomila* and *Matricaria recutita*, respectively, decreased significantly as a result of drought stress.

The strategy of early flowering and maturity is a useful drought escape mechanism, however, it can have a negative effect on the potential grain yield (Fischer 1979; Turner 1986; Bidinger and Witcombe 1989) due to the reduction in available time for photosynthesis and accumulation of seed nutrients required for higher yield (Ludlow 1989; Radhika and Thind 2014).

According to Naik *et al.* (2020), hydrogel has been shown to enhance cell division and elongation and improved plant growth and development, including increased height, number of branches, leaf area index, biomass, and root growth.

2.2 Effect of drought on physiological parameters

Makbul *et al.* (2011) discovered that soybean plants exhibited a decrease in stomatal activity during a period of drought. As compared to normal leaves, leaves exposed to drought exhibited a decrease in stomatal conductance of up to 42%. The drought also influenced the chlorophyll content. The significant statistical importance of this decline in stomatal behaviour and chlorophyll content cannot be ignored.

According to Sircelj *et al.* (2007), the effects of extreme drought on apple trees differ from those of moderate drought, with the former exhibiting a significantly negative impact on the trees' overall health. Furthermore, the cultivars exposed to severe drought demonstrated a reduction in stomatal conductance and transpiration, leading to a decline in net photosynthesis. This decrease was attributed to damage or downregulation of the photosynthetic system, as evidenced by the accumulation of intercellular CO₂, which could not be attributed to a lack of CO₂.

Under conditions of drought stress, cell turgor pressure is notably lowered. This, in turn, results in reduced stomatal activity and photosynthesis, according to Sullivan and Ross (1979). According to Ma *et al.* (2001), water shortage leads to the stomata closer and, thus, a reduction in the photosynthetic rate. While transpiration takes place mostly through the stomata, it also occurs partially through the cuticle. Fariduddin *et al.* (2009) discovered that stomatal conductance was significantly reduced by water stress, regardless of the plant's exposure to drought stress.

According to Abid *et al.* (2018), prolonged exposure to drought conditions resulted in a significant decrease in the stomatal conductance and photosynthetic rate of plants, as compared to those that were well-watered during the same period. Furthermore, the lack of water also led to a decrease in Ψ_w , Ψ_s and RWC, but an increase in osmotic adjustment, as opposed to the well-watered plants. In times of drought, the sensitive cultivar exhibited lower leaf water potential and OA magnitude compared to the tolerant cultivar.

The level of water within a plant is defined by the relative water content of its leaves. Thus, water stress can hinder growth by affecting this factor of the plant. Maintaining a high leaf water balance during drought stress is crucial for a plant's survival, and the relative water content of a plant serves as an important indicator of its ability to withstand drought. The amount of RWC present in potatoes decreased with increasing levels of drought stress at varying growth stages, according to van Loon's study in 1981. According to Gowda *et al.* (1990), water stress caused a reduction in groundnut's relative water content, which was further exacerbated by the age of the plant.

The leaf's RWC is a crucial indicator of a plant's drought tolerance in rapeseed and mustard, according to the findings of Dogra *et al.* (2018). The study involved cultivating different varieties of these crops using various irrigation techniques, and the relative water content value decreased with each replenishing irrigation. The relative water content values of both drought-resistant and drought-prone varieties were reduced, but the latter suffered the most significant reduction. On the 90th day after sowing, the cultivar 'Kranti' recorded the highest mean RWC, while 'Pusa Bold' had the lowest.

Plant physiological activity, including growth and development, can be accurately measured through water metabolism, as noted by Chen *et al.* in 2011. Blum *et al.*, 1999 stated that during pre-flowering drought stress, cultivars with strong genetic variations in osmotic adjustment (OA) yielded better results compared to low OA cultivars. Additionally, there is a positive correlation between OA and both biomass and grain yield. According to Khan's research in 1999, barley varieties have been found to exhibit drought tolerance mechanisms such as the accumulation of metabolites for osmotic adjustment and the conservation of turgor pressure by reducing Ψ s at lower leaf water levels.

During periods of drought, the F_v/F_0 and F_v/F_m values showed a decline of 13.07% and 2.65% for the w-14 cultivar, and 14.92% and 2.35% for the w-20 cultivar, respectively. In situations where the plants received adequate irrigation, the application of Silocon did not influence chlorophyll fluorescence parameters, which include F_v/F_0 and F_v/F_m . This was also found to be true for the chlorophyll content, as noted by Chen *et al.* (2010). In a study conducted by Gadallah (1995), the effect of dehydration stress on CSI and chlorophyll content in cotton crops was examined. According to the findings, the stability of plant membranes under water-stressed conditions was lower compared to that of normal plants. According to Dogra *et al.* (2018), it was observed that varieties with higher CSI had greater tolerance towards drought when compared to plants that were not subjected to stress. Furthermore, it was found that the CSI decreased in all varieties when subjected to drought stress at different DAS, with values ranging from 68.32 % to 54.11 %. Agati *et al.* (2012) found that drought-sensitive inbreds experience a decrease in chlorophyll, while drought-tolerant inbreds show an increase in response to water deficit. This suggests that the photosynthetic components in drought-sensitive lines may be vulnerable to damage, whereas drought-tolerant lines may have the ability to withstand or prevent damage caused by water stress.

According to Selmar's (2008) report, flavonoids, which are a type of specialized secondary metabolites that include flavonols and anthocyanins, are known for their potent radical scavenging ability and have been found to help plants cope with oxidative and drought stress. When subjected to water deficiency, the researchers observed a divergent pattern in the relative flavonol levels between the drought-tolerant and drought-sensitive lines. Specifically, there was an increase in flavonoids in the former and a decrease in the latter. These findings

are consistent with previous research that demonstrates plants under duress tend to produce a higher quantity of flavonoids than those not under stress (Peng *et al.*, 2008). The enhanced flavonoids in the drought-sensitive lines suggest a trade-off between transpiration and photosynthesis. To conserve water, the stoma closes, which impedes the diffusion of atmospheric CO₂ to the leaves for carbon fixation. Therefore, drought-sensitive inbreds have constrained productivity due to their need to retain water. When plants are exposed to a lack of water, a corresponding scarcity of nitrogen will hinder their ability to perform photosynthesis. This nitrogen deficit also reduces the efficiency of the process and leads to a reduction in the enzyme level associated with the Calvin cycle. This results in the buildup of foliar anthocyanin in the leaves of many plant species, as noted by Steyn *et al.* in 2002. Specifically, Miller *et al.* (2009) observed that Anthocyanin levels significantly increased in drought-sensitive 2, which had the greatest reductions in both chlorophyll and NBI. This suggests that the lack of nitrogen caused by the water deficit may have stimulated the activation of gene-encoding enzymes involved in anthocyanin biosynthesis.

2.3 Effect of drought on biochemical parameters

Plants have evolved antioxidant defence mechanisms to combat oxidative stress, which can be divided into two categories. The first class (non-enzymatic) comprise small molecules such as glutathione, vitamins (A, C, and E), carotenoids, and phenols that can directly react with and scavenge ROS. The second class of enzymes includes SOD, POX, GR and CAT. They both represent the most effective defence mechanisms against oxidative stress, capable of removing superoxide and hydrogen peroxide (Miller *et al.*, 2009).

Proline is the most dominant osmoprotectant. In many plants, stress-dependent accumulation of proline has been observed (Yoshida *et al.*, 1997). The accumulation of proline is believed one of the hallmarks of the adaptive stress response (Dalauney and Verma, 1993). Elevated proline levels in cabbage stress are consistent with Hsu *et al.* (2003); Gunes *et al.* (2008) and Din *et al.* (2011).

Turkan *et al.* (2005) observed more proline levels in leaves of tolerant legume cultivars than susceptible cultivars under low humidity conditions. Proline acts as a fine-tuner under stressful conditions, as a metabolic substrate in rapidly dividing cells, and as a cue, a signalling molecule to improved developmental processes such as flowering transition. Lehman *et al.* (2010) provided convincing evidence that it serves as an energy source for rapidly dividing cells during flowering, embryonic and other developmental processes.

According to Dogra *et al.* (2018), under stress conditions such as drought, salinity, and high temperature, metabolites such as proline in plants are highly accumulated. Mustard leaf proline levels were significantly increased in all varieties. The data revealed a notable difference in leaf proline content between the control and 90 DAS from drought stress. Proline content was observed highest in Kranti, followed by 'Bt RSPR-03' and 'RSPR-01',

while 'NRCD-2' and 'Pusa Bold' had the lowest proline content under drought stress at 90 DAS. Under irrigated conditions, the same results were observed at different developmental stages, however, the stressed plants had higher proline levels, and leaf proline levels decreased significantly after watering.

Abid *et al.* (2018) observed a reduction in soluble protein concentration and an improvement in free amino acid and proline concentrations under drought stress. Greater increases in amino acids and proline were observed with severe stress treatment than with moderate stress treatment, and adversely, greater decreases in soluble protein content.

Glycine-Betaine (GB) synthesis depends on individual plant species. For example, barley and spinach accumulate large amounts of GB in their chloroplasts, whereas tobacco and Arabidopsis do not synthesize GB (Sakamoto and Murata, 2002). Chen *et al.* (2007) found that barley varieties showed GB accumulation even under salinity stress of 320 mM.

In nature, proline and GB are sensitive to changes in osmotic pressure and protect subcellular structures in stressed plants (Ashraf and Foolad, 2007). Giri (2011) reported that GB biosynthetic genes improved the resistance of transgenic plants to abiotic stress.

Mansoori *et al.* (2015) observed that GB concentrations were reduced in the leaves of legume plants under moderate water stress conditions compared to irrigated plants. The reduction of GB concentration may be related to the sensitivity of plants to water stress. GB has been reported to be positively correlated with abiotic stress tolerance. However, not all plant species naturally produce or accumulate GB in response to abiotic stresses. According to Moharramnejad *et al.* (2015) GB concentrations in leaves of two maize inbred lines (B₇₃ and MO₁₇) increased under drought stress.

Soluble sugars function not only as metabolic tools but also as signals to control various processes related to plant growth and development (Loreti *et al.*, 2001; Gupta and Kaur, 2005). Hexose and sucrose also play a dual role in the regulation of genes, as evidenced by the downregulation of stress-related genes and the upregulation of growth-related genes (Rosa *et al.*, 2009).

Al-Hakimi *et al.* (1997) observed that soluble sugar content in durum wheat seedlings was a better predictor of drought tolerance than proline content. Mohammadkhani and Heidari (2008) showed an increase (1.18 to 1.90-fold) in soluble sugar content in maize sprouts and roots under drought stress. Starch content decreased significantly, but proline levels increased, suggesting their role in osmoregulation and minimization of dehydration damage. Similar effects were observed in sugar beets and strawberries following the foliar application of algal extracts under drought stress (Blunden *et al.*, 1979; Abo Sedera *et al.*, 2010).

Drought stress resulted in increased total soluble sugar (TSS) and fructose accumulation compared to well-watered conditions. The increase of TSS and fructose was higher in severe stress treatment than in moderate stress treatment (Abid *et al.*, 2018).

The concentration of H₂O₂ in plant tissues under stress conditions is an important factor as it can be a precursor to highly reactive oxygen species (ROS). Higher activity of antioxidant enzymes was observed when low H₂O₂ levels in cells were maintained under drought conditions (Gong *et al.*, 2005). Ma *et al.* (2016) showed that H₂O₂ content was increased in drought-stressed plants compared to well-irrigated treatments.

The concentration of H₂O₂ in plant tissues under stress conditions is an important factor as it can be a precursor to highly reactive oxygen species (ROS). Higher activity of antioxidant enzymes was observed when low levels of H₂O₂ in cells were maintained under drought conditions (Syeed *et al.*, 2011).

2.4 Effect of drought on membrane injury indices

Water is required to maintain membrane fluidity, and under stress conditions, phospholipid bilayers induce different water potentials in different tissues, bringing about structural changes (Blum, 2011). In response to these changes, plants adjust membrane structure in response to water flow out of the cell (Chaves *et al.*, 2003). Under drought conditions, tissue dehydration impairs the plant's ability to maintain membrane fluidity within an optimal range. Relative stress damage and lipid peroxidation are two indicators of membrane damage discussed here.

Drought stress resulted in decreased membrane stability index (MSI) and increased membrane injury (MI). The reduction in MSI was greater in plants under severe stress than in moderate stress treatments and was more pronounced at the onset of stress than at the tillering stage. Tolerant plants maintained a significantly higher MSI during stress and exhibited lower MI compared to responsive varieties (Abid *et al.*, 2018). After rewatering, MSI and MI in moderately stressed plants gradually recovered by 3 days after rewatering to well-watered levels, while severe stress plants experienced incomplete recovery. MSI and MI showed similar recovery trends regardless of the growth stage at which stress was applied (Abid *et al.*, 2018).

H₂O₂ and MDA levels increased rapidly in severely stressed plants and more modestly in moderately stressed plants than in well-watered plants. Drought-stressed plants of sensitive varieties contained higher levels of H₂O₂ and MDA than plants of tolerant varieties, regardless of the growth stage. After re-watering, the concentrations of H₂O₂ and MDA decreased rapidly, especially in moderately stressed plants, reaching levels comparable to those of well-watered plants. In the severely stressed plant, H₂O₂ and MDA levels never completely decreased to those of the well-watered plant, even after rewatering (Abid *et al.*, 2018).

2.5 Effect of drought on antioxidant defence system

High Superoxide dismutase (SOD), Catalase (CAT), and Ascorbate peroxidase (APX) activities were associated with reduced malondialdehyde (MDA) content in wheat at different water capacities (Ezzat-Ollah *et al.*, 2007).

Habibi (2014) showed that the activity of SOD enzyme in shoots and roots increased

with mild water stress (15 days after water retention), but gradually decreased as water stress became more severe. An increase in SOD activity was observed 18 to 25 days after water stress but decreased significantly after 20 to 25 days of water stress. The application of silicon resulted in a significant increase in the activity of all antioxidant enzymes such as SOD.

Biju *et al.* (2017) showed that the activities of antioxidant enzymes such as APX, CAT and SOD were significantly increased under drought stress compared with normal lentil seedlings.

Catalase (CAT), a well-known metalloenzyme, is one of the most powerful protein catalysts known. This is a non-chloroplast enzyme abundant in plant tissues that detoxifies H₂O₂ directly into water and oxygen. Its strongest catalytic activity is associated with peroxisomes, where H₂O₂ produced during photorespiration is removed (Foryer and Mullineaux, 1994).

Ascorbate peroxidase (APX) scavenges peroxidase by converting ascorbic acid to dehydroascorbate. It is part of the ascorbate-glutathione cycle and plays a vital role in the removal of toxic H₂O₂ from plant cells (Ozkur *et al.*, 2009).

In plants not treated with salicylic acid, drought stress significantly increased leaf tissue SOD activity compared with controls. However, these increases were more pronounced in the *Baharband* variety than in the other ecotypes (42.57%) under drought stress. Among the drought-stressed plants, the *Fetrezamin* and *Gavdareh* varieties had the lowest SOD activities, 20.8 (mg protein⁻¹) and 20.9 (mg protein⁻¹), respectively. Furthermore, the increase in SOD activity was consistently accompanied by a significant increase in CAT activity in leaves of drought-treated ecotypes compared to the control, with the lowest CAT activity level in cultivar *Gavdareh* and the highest value in cultivar *Baharband* (Khalvandi *et al.*, 2021).

In wheat (*Triticum aestivum* L.), Abid *et al.* (2018) observed that drought stress increased the enzyme activities of CAT, SOD and APX. According to Lou *et al.* (2018) under water-deficient conditions, the GSH/GSSG ratio increased by 64.2% in flag leaves compared to well-watered treatment conditions, while it increased by 39.6% and 54.8% in glumes and lemmas, respectively. In contrast to these results, the ASA/DHA ratios of flag leaves and husks gradually decreased during the grain-filling stage, and the ASA/DHA ratios in flag leaves decreased by 51.6% under the well-watered condition, whereas this was the case for glumes and lemmas decreased by 43.2% and 35.8%, respectively. It was found that the GSH/GSSG ratio and ASA/DHA ratio were more efficiently maintained in glumes and lemmas compared to flag leaves. These results suggest that the role of the spike organ in balancing the redox status of ascorbic acid and glutathione under water stress cannot be ignored.

2.6 Effect of drought on yield and yield attributes

According to Singh *et al.* (2017), both plant height and dry matter accumulation were affected by the irrigation schedule and hydrogel application. The application of irrigation was

still significantly better than no irrigation in terms of plant height and dry matter accumulation. However, Irrigation water/Cumulative pan evaporation planning at different levels remained statistically the same due to high rainfall and low evaporation rates during the growing season. Increases in plant height are due to irrigation water supply at critical stages providing a comfortable growth environment, enhancing cell elongation, cell swelling, stomatal opening, and ultimately efficient distribution of photosynthesis into the sink (Chauhan *et al.*, 2002). The plant height is short, which may be caused by water stress during the critical period of water demand. The use of superabsorbents enhances all the agronomic characteristics of the crop (Moghadam *et al.*, 2009). Greater accumulation of dry matter from irrigation and hydrogel application may be due to better growth and developmental conditions promoted by the required water supply during critical periods, thereby increasing plant height and the number of branches per plant. Yield attributes, i.e. primary and secondary branches per plant, siliques per plant and 1000 seed weight were significantly affected by irrigation and hydrogel application under rainy conditions. Irrigation significantly increased the number of branches per plant, the number of pods per plant, and the weight of 1000 seeds compared to no irrigation. However, irrigation schedules based on Irrigation water/Cumulative pan evaporation ratios were not significantly different for the reasons mentioned above. These results are broadly consistent with those of Sharma (1994). This may be due to better water availability, which favours branch development by maintaining a better water status. Irrigation and hydrogel application not only boosted plant growth and development but also provided a greater supply of nutrients, leading to more branching and better gutter development, resulting in a higher number of pods per plant. The higher 1000-seed weight could be due to better nutrient availability and better transfer of photosynthesis from source to sink, which in turn facilitated higher photosynthetic accumulation in seeds through irrigation and hydrogel application. Similar results were reported by Padmini *et al.* (1994) and Yadav *et al.* (2010).

Drought stress negatively affects plant height in all wheat varieties, regardless of cultivar or cultivar (Kilic and Yagbasanlar, 2010). However, the drought period is the most severe, followed by water loss, which eventually leads to a reduction in plant height (Nonami, 1998; Oladiret *et al.*, 1999; Hussain *et al.*, 2008; Khakwani *et al.*, 2012) and panicle length. However, it is ambiguously influenced by tiller drought, pre-anthesis drought, and post-anthesis drought (Saleem, 2003; Mirbahar *et al.*, 2009). When exposed to drought stress from the single-leaf stage to the flowering stage, wheat height is significantly affected resulting in low dry matter accumulation and ultimately reduced plant yield (Moayedi *et al.*, 2010). Protoplasts become dehydrated and lose their expansibility during drought, impairing cell elongation, expansion and mitosis. In addition, plants shed their leaves to prevent water loss, which eventually leads to reduced plant height (Nonami, 1998; Oladiret *et al.*, 1999; Hussain *et al.*, 2008; Khakwaniet *et al.*, 2012;).

According to Khatiwada *et al.* (2020), drought stress affects plant height and plant biomass, number of spikes, number of spikelets and final grain yield. Although all yield components of all wheat varieties were negatively affected by drought, the magnitude of the effects varied. The extent to which drought stress affects crops mainly depends on the following parameters: phenological phase of the crop, intensity and duration of drought stress. The response of wheat to drought stress may also be affected by plant morphological characteristics, such as the number of effective fertile buds per plant, the number of spikes, the number of spikelets per spike, the number of grains per spike, bulk density, spike length, and spike weight and biomass. If a drought occurs during harvest or flowering, crop yields will be greatly reduced.

2.7 Effect of drought on soil moisture studies

Soil moisture is the amount of water stored in the soil that can be taken up by plants along with minerals. Insufficient soil moisture reduces the stomatal conductivity of plants. When soil moisture is depleted, the water-holding capacity of plants decreases, causing stomata to close and transpiration rates to decrease, ultimately leading to dry matter accumulation and reduced yields. These results suggest that soil water scarcity affects internal electrical conductivity, whereas environmental water scarcity does not (Woldeamanuel *et al.*, 2005).

When water availability for irrigation is limited, the use of synthetic polymers can increase water availability to plants by restricting water flow beyond the root zone (Narjary *et al.*, 2012). These polymers absorb stored rainwater and other moisture, releasing them gradually at a later stage to meet crop water needs and extend irrigation intervals (El-Hady and Camilia, 2006). Hydrogels reduce nutrient loss by preventing leaching, especially nitrogen and potassium leaching. Thus, it facilitates the synchrony of nutrient release and uptake according to crop needs (Pimoradian *et al.*, 2004).

2.8 Role of salicylic acid in mitigating the adverse effect of drought

Salicylic acid (SA) is an oxidative plant growth regulator that, at least in small amounts, plays an important role in plant protection systems against biotic and abiotic stresses. SA not only has a positive effect on plant growth and development under water stress conditions but also has a positive effect on normal water supply. In this regard, SA is one of the most important plant growth regulators, playing a crucial role in the control and regulation of photosynthesis under normal and stressful conditions (Arif *et al.*, 2020). SA plays a key role in enhancing photosynthesis by upregulating photosynthetic enzymes and carbohydrate metabolism (Khodary, 2004). SA application increased photosynthetic activity, chlorophyll content, and enzyme activity under normal and stressful conditions (Li *et al.*, 2014). Consequently, the total levels of chlorophyll and carotenoids increased, while proline levels decreased with SA intake at each irrigation level. Such results could be reflected and explained, at least in part, by improved sunflower growth and sunflower productivity due to

the exogenous application of SA. The improvement of sunflower growth by SA application can be attributed to the role of SA in maintaining photosynthetic machinery and activity, thereby promoting plant growth. SA promotes various physiological processes such as photosynthesis, chlorophyll and other pigments, plant growth and development, and flowering (Arif *et al.*, 2020). SA plays a key role in ensuring tolerance to plants undergoing water stress, viz. Drought or flood (Hayat *et al.*, 2010). Exogenous application of SA promotes growth; and flowering; upregulates photosynthesis; and increases enzymatic and nonenzymatic antioxidant activity (Arif *et al.*, 2020). Furthermore, ROS production during drought stress requires the upregulation of detoxification systems such SOD and CAT, as well as the biosynthesis of ROS scavengers (You and Chan, 2015). In doing so, SA increases the activity of antioxidant enzyme systems, i.e. APX and SOD, along with decreased CAT, protecting and enhancing nitrate-metabolizing enzymes in stressful environments (Hayat *et al.*, 2010). Furthermore, SA increases the activity of ROS-scavenging enzymes and is involved in triggering abiotic stress responses such as drought (Arif *et al.*, 2020). Application of SA to drought-stressed plants resulted in growth restoration, increased photosynthesis, and reduced oxidative stress (Zulfiqar *et al.*, 2021). Thus, foliar application of SA increased leaf area, pellet diameter, seed yield per capita, seed index, and seed yield ha⁻¹. After the drought, stomata gradually close, leading to a parallel decline in net photosynthesis and water use efficiency (Anjum *et al.*, 2011). Furthermore, different SA concentrations increased leaf total chlorophyll content, photosynthesis, and stomatal conductance under normal and stress conditions (Bastam *et al.*, 2012; Ghasemzadeh and Jaafar 2013). SA stimulates CO₂ fixation and photosynthetic quantum efficiency (Poór *et al.*, 2011). SA also helps facilitate the photosynthetic process by closing stomata and inhibiting or slowing down PSII electron transport metabolism (Janda *et al.*, 2012). Photosynthesis, stomatal conductance, gas exchange, CO₂ assimilation rate, and chlorophyll content were improved after SA application (Babar *et al.*, 2014). SA increases the photosynthesis rate by promoting the carboxylation rate, chlorophyll amount (i.e. SPAD value) and increasing turgor pressure (Tahjib–Ul–Arif *et al.*, 2018). Furthermore, SA increased the levels of chlorophyll, carotenoids, nitrogen, potassium, and phosphorus, and the activity of nitrate reductase (Hashmi *et al.*, 2012). It also maintains the membrane integrity of chloroplast membranes (Huang *et al.*, 2016).

2.9 Role of hydrogel in mitigating the adverse effect of drought

Hydrogels are one way to increase water availability and water use efficiency (Yu *et al.*, 2011; Liao *et al.*, 2016; El-Asmar *et al.*, 2017). Once hydrogels come into contact with water, they become gels that can absorb 400 to 1600 times their original weight in water (Ahmed, 2015; Suresh *et al.*, 2018). The amount used depends on the molecular weight, formation and structure of the hydrogel (Riad *et al.*, 2018). Farmers use hydrogels as water-retaining agents to increase soil available water content, which is critical for plant growth and

crop yields (Yu *et al.*, 2012; Liao *et al.*, 2016; Abrisham *et al.*, 2018; Guo *et al.*, 2020). Hydrogels used in agriculture are mainly composed of polyacrylamide with acrylic acid as the basic unit (Bai *et al.*, 2010). More recently, hydrogels have been constructed with cellulose (Mi *et al.*, 2018), proteins (Kong *et al.*, 2019), and starch (Mahmoodi-Babolan *et al.*, 2019). In addition to the benefits of increasing water availability, hydrogels can also improve the physical, chemical and biological properties of soils, especially in arid and semi-arid regions with infrequent rainfall, irregular long dry periods and high evaporation (Hüttermann *et al.*, 2009; Agaba *et al.*, 2010; Riad *et al.*, 2018; Xu *et al.*, 2018; Guo *et al.*, 2020). Many studies have shown the effect of hydrogels on increasing water use efficiency. They increase yield and plant survival under drought stress and prolonged drought, and reduce watering frequency by increasing water availability to plants (Hüttermann *et al.*, 2009; Nazarli *et al.*, 2010; Islam *et al.*, 2011; Demitri *et al.*, 2013; Jain *et al.*, 2017; Kargar *et al.*, 2017).

Jat *et al.* (2018) observed that different irrigation schedules and hydrogel levels significantly affected the growth of mustard plants, namely plant height. Below 0.8 Irrigation water/Cumulative pan evaporation ratio, significantly higher plant heights were observed, compared to the rest of the treatments.

Record growth attributes such as plant height and multiple branches of plant⁻¹ at the maximum growth stage of 90 DAS. The data showed that at different hydrogel dosages, plant height (106 cm) and the number of branches (3.4) were significantly higher in the treatment applying 100% recommended hydrogel dosage to the soil than in the other treatments. Hydrogels have been reported to increase the activity of cell division, cell expansion, and cell elongation, ultimately resulting in increased plant height, branch number, leaf area index, plant biomass, and root growth (Naik *et al.*, 2020).

The highest RWC was recorded when the hydrogel was irrigated with a hydrogel with an Irrigation water/Cumulative pan evaporation (IW/CPE) ratio of 0.8 during the pre-anthesis and pod development stages. Similar RWC with scheduled irrigation of 0.8 IW/CPE-HG (without hydrogel) and 0.6 IW/CPE + HG (with hydrogel) indicated that the hydrogel response was poorer at higher irrigation regimes. Among other irrigation systems, hydrogels significantly improved RWC. RWC at the pod development stage had a significantly higher impact on seed yield than at the pre-anthesis stage. The RWC of leaves irrigated with 0.6 IW/CPE-HG hydrogel was at the same level as that of 0.4 IW/CPE+HG. Likewise, rainfed crops +HG and plants irrigated with 0.4 IW/CPE -HG showed similar leaf RWC (Rathore *et al.*, 2020).

Growth traits were highest, and yield-related traits were highest due to increased photosynthetic activity and translocation of photosynthesis from source to sink. These results are broadly consistent with the findings of Singh *et al.* (2014). It turns out that using hydrogels is much more efficient than not using them. However, the weight of 1000 seeds was not significantly affected due to the different hydrogel contents. Generate more yield-

related traits, i.e. Number of primary branches/plant, number of pods/plant, number of seeds/bones and pod length when using 5.0 kg/ha compared to no hydrogel, statistical results compared to using 2.5 kg/ha hydrogel same. This is likely because the hydrogel absorbs at least 400 times the dry weight of pure water and releases it gradually based on the needs of the plants. In addition, it also has the physical properties of soil, namely, Porosity, soil permeability and soil infiltration improve soils by increasing water holding capacity (Narjary *et al.*, 2013) and efficient use of soil-bound water (Choudhary *et al.*, 2014), which help plants withstand long-term water stress Prevents the onset of permanent wilting and reduces the watering needs of plants as less water is lost to evaporation. All these beneficial aspects of the hydrogel resulted in a significant increase in seed yield (1952 kg/ha) when applied at 5.0 kg/ha. Application of 5.0 kg/ha hydrogel resulted in a corresponding increase in mustard seed yield of 5 % and 8.86% compared to 2.5 kg/ha and anhydrous gel, respectively.

Soil moisture was maintained at the highest level before flowering and pod setting, with a watering frequency of 0.8 IW/CPE, with and without hydrogel. Due to the water-absorbing nature of hydrogel, irrigation at 0.6 and 0.4 IW/CPE + HG saved 13.6%, 27.3%, and 15.9% of irrigation water in 2013-14 and 2014-15 compared to 0.8 IW/CPE + HG, respectively (Rathore *et al.*, 2020).

Sheikh *et al.* (2020) showed in their experimental results that all soil amendments significantly increased the water-holding capacity of the soil. However, the hydrogel, FYM, compost, and gypsum treatments increased water holding capacity by 63.01, 37.20, 43.75, and 35.71%, respectively, compared to the control group. The comparison between soil amendments showed that the hydrogel increased the water-holding capacity of the soil by 41.09, 34.24 and 42.46% compared with FYM, compost and gypsum, respectively.

2.10 Role of gond katira in mitigating the adverse effect of drought

Recently, Kanal Regional Station ICAR-IARI discovered a new environmentally friendly and low-cost (300/kg) herbal hydrogel "Katira-Gel" (dried sap of the plant *Tragacanth* sp., known in humans as E- 413) for food labelling in the international market, known locally as "Gond Katira" and developed a farmer-friendly seed primer using hydrogel coating technology for direct seeded rice, wheat, pulses, oilseeds and other crops to achieve efficient hydrogel delivery systems to the root zone, thereby mitigating by saving water and making agriculture more sustainable and resilient to climate change, improving seedling survivability and increasing water efficiency Suboptimal water stress.

The water absorption capacity of the herbal hydrogel "Katira Gel" is comparable to that of the commercially available Pusa hydrogel, i.e. at room temp., it weighs about 100 times its weight in ordinary tap water. According to Nibhoria and Kumar (2022), the effect of *Astragalus* on plant height of 30, 60, 90 and 120 DAS is consistent with the findings of Kumar *et al.* (2019) and agree with Lather (2019) Kumar and Singh (2020) at Harvest. The

increased plant height of SA may be due to its role in plant systems as a phenolic phytohormone and a signalling molecule that induces specific changes in leaf anatomy and enhances ion uptake, thereby promoting plant growth (Pandey *et al.*, 2020).

For agrochemicals, higher shoot dry matter accumulation differed significantly in barley, with maximum treatment deficits for Astragalus and SA occurring at 30 and 60 DAS. This may be due to the water-holding capacity of Astragalus katira and the higher availability of water in the root zone when the plant needs it. The combination of Astragalus and SA resulted in shoot dry weights that were 54.6%, 64.0% and 58.5% higher than the control at 90 and 120 DAS and harvested. This can be attributed to the fact that both the vegetative and reproductive stages of plants are facilitated by adequate water. Stress at any stage of plant growth can shorten a plant's life cycle by a few days, as they tend to finish it early to avoid or escape unfavourable stress conditions. Similar results were also reported by Hussien *et al.* (2019) in Mung Bean and Muleke *et al.* (2022) in Wheat. No significant effect of agrochemicals was observed on days to 50% flowering, whereas days to physiological maturity were delayed by 4 to 5 days after treatment with Tragacanth katira and SA. It shows the effect of SA on stomatal conductance and moisture uptake.

Kumar *et al.* (2019) conducted a two-year study in Durgapur to investigate the effects of different irrigation levels and hydrogels on the HD 2967 wheat variety. Two hydrogels, herbal hydrogel (tragacanth, i.e. gondkatira) at a rate of 400 ml/100 kg seeds and Pusa hydrogel at a rate of 2.5 kg ha⁻¹ were used for seed treatment and soil application, respectively, and served as controls (without treatment). The herbal hydrogel was similar to the control (without seed treatment) in terms of plant height, plant growth rate, and dry matter accumulation at the initial stage of culture, but significantly higher growth parameters than the control were observed near maturity. Wairagade *et al.* (2020) also reported similar results. Rathore *et al.* (2020) reported an increase in RWC content when the hydrogel was applied to soil compared to a control of Indian mustard under irrigation and rain conditions of 0.8, 0.6 and 0.4 IW/CPE.

CHAPTER-III

MATERIALS AND METHODS

The details of materials used and experimental methodology followed during the present study are described in this chapter as follows:

3.1 Experimental site

The present investigation entitled “**Enhancing physiological efficiency and yield potential of Indian mustard using hydrogel and salicylic acid under rainfed condition**” was conducted at the Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar, India. The crop was grown in the research farm of Oilseeds Section Department of Genetics and Plant Breeding, College of Agriculture, CCS Haryana Agricultural University, Hisar (Haryana) during two successive *rabi* seasons of 2019-20 and 2020-21 which is situated at a latitude of 29° 10'n and longitude of 75° 46' e an altitude of 215.2 m above the main sea level and falls in the semi-tropical region of western zone of India. the soil showed a sandy loam texture, it was alkaline with a pH of 8.1-8.4 and EC of 0.22-0.57dsm⁻¹. Analytical work was done at the stress physiology laboratory of the Department of Botany and Plant Physiology. The weekly averaged meteorological data at CCS Haryana Agricultural University, Hisar during the *Rabi* season of 2019-20 and 2020-21 is given in Fig. 1.

3.2 Chemicals used: In the present investigation, all the chemicals used were of analytical grade (AR) & were procured from Sigma chemicals Co., USA, Hi-Media, Sisco research laboratories & E. Merck.

3.3 Experimental material: The seeds of Indian mustard [*Brassica juncea* (L.) Czern & Coss] variety RH 725 was procured from the Oilseed Section, Department of Genetics and Plant Breeding, College of Agriculture, CCS Haryana Agricultural University, Hisar (Haryana).

3.3.1 Crop raising:

The crop was grown in two successive years (2019-20 and 2020-21). Seeds were mixed with different levels of hydrogel (3.75kg/ha and 5kg/ha) and gond katira (15kg/ha and 20kg/ha) according to respective treatment. Seeds were grown in a randomized block design (RBD) with 3 replications at the research farm of the Oilseeds Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar. The plots size of 9m² in each replication with a spacing of 30 cm between the rows and 10 cm plant to plant distance.

3.3.2 Date of sowing: 15th October (2019-20) and 16th October (2020-21)

3.3.3 Foliar spray:

The crop was sprayed with different concentrations of salicylic acid (SA @ 100ppm and 200ppm) with the help of a manual sprayer at the flowering (50 DAS) and siliqua formation stage (60 DAS). For the preparation of different levels of salicylic acid (100ppm and 200ppm), the chemical was first dissolved in 100 µl ethanol and then diluted to the required volume with distilled water.

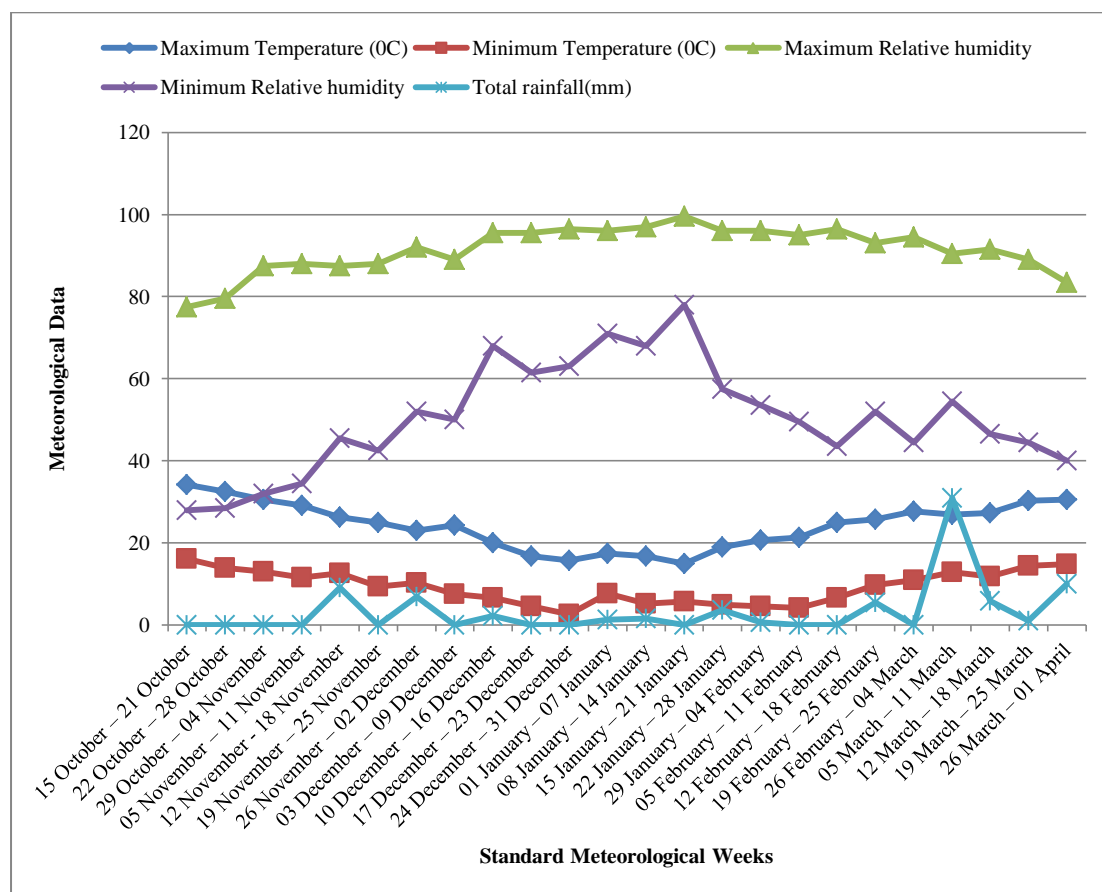


Fig 1: Graphical representation of weather parameters during Rabi crop season of 2019-20 and 2020-21 at CCS Haryana Agricultural University, Hisar

3.3.4 Treatments:

- T₁ Control (Rainfed with no hydrogel and salicylic acid)
- T₂ Hydrogel (3.75kg/ha)
- T₃ Hydrogel (5kg/ha)
- T₄ Gond Katira (15kg/ha)
- T₅ Gond Katira (20kg/ha)
- T₆ Salicylic acid (100ppm)
- T₇ Salicylic acid (200ppm)
- T₈ Hydrogel (3.75 kg/ha) + SA (100ppm)
- T₉ Hydrogel (3.75kg/ha) + SA (200ppm)

T₁₀	Hydrogel (5kg/ha) + SA (100ppm)
T₁₁	Hydrogel (5kg/ha) + SA (200ppm)
T₁₂	Gond Katira (15kg/ha) + SA (100ppm)
T₁₃	Gond Katira (15kg/ha) + SA (200ppm)
T₁₄	Gond Katira (20kg/ha) + SA (100ppm)
T₁₅	Gond Katira (20kg/ha) + SA (200ppm)
T₁₆	Irrigated

3.3.5 Sampling: For each parameter, sampling was done five days after the spray of SA (i.e. at flowering and siliqua formation stage) from three replications.

3.3.6 Statistical design: Data were analyzed using randomized block design (one-factor analysis of variance and expressed as mean values \pm SE). Treatments were compared using critical difference (CD) at a 5% level of significance with the online statistical analysis package “OPSTAT” (Sheoran *et al.*, 1998). Treatment grouping was done with the online statistical analysis package “GRAPES (general R shiny based analysis platform empowered by statistics) (Gopinath *et al.*, 2020).

3.4 Observations:

The following observations were recorded:

3.4.1 Growth and reproductive parameters:

3.4.1.1 Days to 50% flowering: Days to 50% flowering were measured from the date of sowing to when 50% of plants in a plot had at least one flower.

3.4.1.2 Days to physiological maturity: These were recorded from the date of sowing to when plants showed physiological maturity stage.

3.4.1.3 Plant height: Plant height was measured from the surface of the sand to the apex of the plant using a meter scale and expressed in cm.

3.4.2 Physiological parameters:

3.4.2.1 Gas-exchange parameters: The parameters related to gas exchange, including the photosynthetic rate (A), transpiration rate (E), and stomatal conductance (gs), were measured using an infrared gas analyzer (IRGA; LCI-SD, ADC Bioscientific Ltd., Hoddesdon, UK). The fully expanded leaves were enclosed in the assimilation chamber between 12:00-2:00 P.M., and repositioned to obtain the maximum photosynthetically active radiation (PAR). The system automatically calculated the values of A, E, and gs based on the preloaded flow rate and leaf area.

3.4.2.2 Water potential (Ψ_w): Between 12:00 and 2:00 P.M., leaf water potential was evaluated utilizing a Model 3005 pressure chamber from Soil Moisture Equipment Corporation located in Santa Barbara, CA, USA. A sharp-edged knife was utilized to cut a fully expanded leaf from the top of the plant, and each leaf was subsequently sealed inside the pressure chamber. The cut end of the leaf was left protruding outside the chamber. The pressure was then applied until the sap appeared at the cut end, and the pressure was recorded for the corresponding tissue. This value was then transformed into the SI unit (-Mpa).

3.4.2.3 Osmotic potential (Ψ_s): To determine the osmotic potential of a plant, leaf samples were obtained from the uppermost part of the plant. These samples were then placed in airtight syringes and frozen at a temperature of -20°C for the time duration of 24 hours. From the frozen leaf tissues, the sap was extracted onto filter paper discs, and Ψ_s was measured using a vapour pressure osmometer with the model number 5100-B, manufactured by Wescor Inc. of Logan, Utah, USA. The osmometer was calibrated with reference standards of sodium chloride from Wescor mc, USA. The readings obtained from the osmometer were in mmol kg^{-1} , which were then converted to molality and further to (-) bars by making use of a calibration curve. This calibration curve was expressed in (-) MPa, and the conversion factor was used:

$$40 \text{ mmol kg}^{-1} = -1 \text{ bar}$$

$$-10 \text{ bar} = -1 \text{ MPa}$$

3.4.2.4 Relative water content (RWC): To obtain samples for analysis, fully expanded leaves were obtained and separated from the shoots during the hours of 9:00-11:00 A.M. These samples were then swiftly placed in polythene bags that were humidified and sealed to maintain their freshness during transportation to the laboratory, which was done on ice. In the laboratory, a soft brush was used to remove any sand present on the leaves. Afterwards, the leaf samples were cut into small, one-square centimetre fragments and weighed immediately to determine their fresh weight. These fragments were then placed in separate petridishes filled with distilled water for no less than four hours. Once fully turgid, the leaf samples were weighed again to determine their turgid weight and were then dried in an oven at 65°C for 72 hours until a constant dry weight was achieved. These three values were utilized to calculate RWC (%), using the formula developed by Weatherly (1950).

$$\text{RWC (\%)} = (\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight}) \times 100$$



Fig. 3: Experimental view of Indian mustard variety RH 725 at research farm of Oilseeds Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar

3.4.2.5 Quantum yield: The OS-30P chlorophyll fluorometer, manufactured by Opti-Science, Inc. in Hudson, USA, was used to determine the photochemical efficiency or quantum yield of intact plants. The machine recorded the initial (F_0) and maximum (F_m) fluorescence and derived variable fluorescence (F_v) by subtracting F_0 from F_m . Quantum yield was then calculated by dividing F_v by F_m ratios.

3.4.2.6 Flavonol, Anthocyanin, and Chlorophyll Indices: The flavonol, anthocyanin, and chlorophyll indices were also measured using a Force-A Dualiex scientific+ device made by FORCE-A in Orsay, France. The measurement was conducted by opening a clip using the handle, placing a leaf between the optical heads, and releasing the handle to automatically take the measurement. Two types of beeps could sound after each measurement: a long beep indicating that the measurement was accurately taken and recorded, and three short beeps indicating that the measurement was incorrect and not recorded. The method used for these measurements was in accordance with Cerovic *et al.*'s (2012) methodology.

3.4.2.7 Chlorophyll stability index: To estimate the overall chlorophyll present, Hiscox and Israelstom's method from 1979 was utilized. An extract was created from 50 mg of leaf sample and placed within an Amber glass vial, containing 5 ml of dimethyl sulphoxide (DMSO). These vials were then heated in an oven, set to 65°C, for the time duration of 4 hours before being allowed to cool to room temperature. The resulting extracts were assessed for their absorbances at 480, 663, and 645 nm using a spectrophotometer, with DMSO serving as a blank. The calculation of chlorophyll content was determined as:

$$\text{Chl a} = [12.7 \times A_{663} - 2.69 \times A_{645}]$$

$$\text{Chl b} = [22.9 \times A_{645} - 4.68 \times A_{663}]$$

$$\text{Chl}_{\text{Total}} = [20.2 \times A_{645} + 8.02 \times A_{663}]$$

The values thus obtained are in $\mu\text{g/ml}$ of extract (solvent). The value of total carotenoid ($\mu\text{g g}^{-1}$) was determined using the following formula:

$$\text{Total carotenoid} = [1000 \times A_{480} - (3.27 \times \text{Chl a} + 104 \times \text{Chl b})] / 229$$

Values in mg/g fresh weight are obtained by multiplying the above values with “ $V/(W \times 1000)$ ”, where V is the volume of extract; W is the fresh weight of the sample.

The chlorophyll stability index (CSI %) was determined as a formula given by Sairam *et al.* (1997).

$$\text{CSI (\%)} = (\text{Total chlorophyll under stress} / \text{Total chlorophyll under control}) \times 100$$

3.4.3 Biochemical parameters:

3.4.3.1 Proline content: Proline content was estimated using the method of Bates *et al.* (1973).

Reagents

- (i) To create a solution of 3% aqueous sulphosalicylic acid (w/v), dissolve 30 grams of sulphosalicylic acid in 500 ml of distilled water. Once mixed, add more distilled water until the final volume reaches 1 litre.

(ii) The acid ninhydrin reagent is produced by combining 1.25 grams of ninhydrin with 30 ml of glacial acetic acid and 20 ml of 6 M ortho-phosphoric acid. To create the 6 M ortho-phosphoric acid, 38.178 ml of 88% ortho-phosphoric acid was mixed with distilled water and the volume was brought up to 100 ml.

(iii) Toluene

Extraction

To obtain the supernatant, 100 mg of fresh leaves weighing were homogenized in 5 ml of 3% sulphosalicylic acid and then centrifuged at 5000 rpm for 15 minutes.

Procedure

In a test tube, two ml of supernatant were collected and mixed with a solution of two ml of acid ninhydrin reagent and two ml of glacial acetic acid. The resulting concoction was then placed in a boiling water bath for one hour before being halted by transferring the tubes to an ice bath. Following this, four ml of toluene was added. After thoroughly shaking the tube, the upper organic layer containing a pinkish-red hue was collected once it reached room temperature. The absorbance was then recorded at 520 nm with toluene serving as the blank.

Using 3% sulphosalicylic acid and graded concentrations of proline, a standard curve was prepared. The proline content was expressed as mg g^{-1} DW.

3.4.3.2 Glycine-Betaine content: Glycine betaine was estimated according to the method of Grieve and Grattan (1983).

Reagents

- (i) 0.05 % Toluene: 500 μl toluene was dissolved in distilled water. After mixing, the final volume was made to 1 L by adding distilled water.
- (ii) 2N HCl: 17.67 ml of 35 % HCl was taken and the make-up volume was to 100 ml by adding distilled water.
- (iii) Potassium tri-iodide solution: 7.5 g iodine and 10 g potassium iodide in 100 ml 1N HCl (prepared by mixing 8.84 ml of 35 % HCl and making volume 100 ml with distilled water).
- (iv) 1,2-dichloroethane

Procedure

To prepare the sample extract, 0.5 grams of fresh leaves were chopped and mixed with 5 ml of a toluene-water mixture that contained 0.05% toluene. The resulting mixture was placed in 20 ml test tubes and mechanically shaken for 24 hours at a temperature of 25°C . After filtration, 0.5 ml of the extracted solution was combined with 1 ml of 2N HCl solution, and 0.1 ml of potassium tri-iodide solution was added. The solution was then shaken in an ice-cold water bath for 90 minutes before adding 2 ml of ice-cold water. Subsequently, 10 ml of 1,2-dichloroethane, which had been chilled to -20°C , was added to the solution. By passing a continuous stream of air for 1-2 minutes, two layers were separated. After 2 hours, the upper

aqueous layer was removed, and the optical density of the pink-coloured organic layer was measured at 365 nm against 1,2 dichloroethane as a blank. A standard curve was prepared using graded concentrations of glycine-betaine, and the data were expressed as mg g⁻¹ DW of the tissue.

3.4.3.3 Total soluble sugar content: To assess the ethanol soluble sugar content of leaf samples, 200 mg of each respective sample was gathered and homogenized in 80% (v/v) ethanol. The resulting homogenate underwent three rounds of centrifugation at 8000 rpm for 10 minutes each. The separated supernatant fractions were combined and brought to a final 5 ml volume using 80% ethanol. The ethanol-soluble sugars were evaluated in the supernatant. Once separated, 0.5 ml of the supernatant was transferred into a separate test tube and dehydrated in an oven at 60°C. The quantification of soluble sugar was carried out using the anthrone reagent method (Bubois *et al.*, 1951). To the dried material, 1 ml of distilled water was added, followed by 5 ml of freshly prepared anthrone reagent, which was created by dissolving 2 g of anthrone in 1000 ml of concentrated H₂SO₄. The tubes were then incubated in a boiling water bath for 10 minutes before cooling.

Using the Eppendorf BioSpectrometer basic spectrophotometer, the brownish to dark green colour's absorbance was measured at 620 nm. Blank was prepared by mixing 1 ml distilled water in 5 ml anthrone reagent. A standard curve was prepared by taking the known amounts of glucose.

3.4.3.4 Hydrogen peroxide content: H₂O₂ content of the leaves was determined by the modified method of Patterson *et al.* (1984).

Reagents

- (i) 5 % trichloroacetic acid (TCA): 50 g trichloroacetic acid was dissolved in distilled water and made volume upto 1 L.
- (ii) 100 mM potassium phosphate buffer (pH 8.4): Potassium hydrogen phosphate (0.1 M) was prepared by dissolving 1.74 g of it in 100 ml dH₂O. Further, potassium dihydrogen phosphate (0.1 M) was prepared by dissolving 1.36 g of it in 100 ml dH₂O. Potassium hydrogen phosphate and potassium dihydrogen phosphate were mixed in the ratio of 61:39 and pH was adjusted to 8.4 with concentrated ammonia solution.
- (iii) Colorimetric reagent:
 - a. 0.6 mM M₄-(2-pyridylazo) resorcinol solution was prepared by mixing 22.97 mg chemical in dH₂O and make volume 150 ml.
 - b. 0.6 mM potassium titanium oxalate solution was prepared by mixing 31.88 mg potassium titanium oxide oxalate di-hydrate in dH₂O and making a volume of 150 ml.
 - c. Mix solution (a) into (b) in equal quantity (1:1 ratio). The mixture was kept on ice until use.

Extraction

To properly process the leaves, 300 mg were mixed with 0.2 g of activated charcoal and

5 ml of 5% TCA. The mixture was homogenized, then put through a whatman filter paper No.1 and finally centrifuged for 10 minutes at 8000 x g.

Procedure

To prepare the sample, 200 µl of the extract was combined with 100 mM potassium phosphate buffer to create a solution of 4 ml in volume. Following this, 2 ml of colorimetric reagent was introduced into the reaction mixture. The orange hue of the resulting mixture was measured at 508 nm against a blank buffer. By comparing the absorbance of the sample to the blank buffer, the concentration of H₂O₂ was determined. Its molar extinction coefficient of 3.6x10⁴ mole⁻¹ cm⁻¹ was used to calculate the amount of H₂O₂ present in moles per gram of dry weight.

3.4.4 Membrane injury indices:

3.4.4.1 Relative stress injury (%): Sullivan (1979) measured the membrane injury index as the percentage of ion leakage into the external aqueous medium in relation to the total ion concentration of the stressed tissue, which was determined by the electrical conductivity (EC) of the external medium.

Procedure

To conduct the experiment, 200 mg of leaf samples were placed into vials containing 10 ml of de-ionized water and kept at a temperature of 40⁰C for 30 minutes. Following this, the electrical conductivity of the solution was recorded and labelled as EC₁. Next, the samples were heated in a boiling water bath for 1 hour to ensure complete tissue destruction. After the samples had cooled, the electrical conductivity of the solution was measured once more and labelled as EC₂. Finally, the relative stress injury was calculated according to the following formula:

$$RSI (\%) = 1 - EC_1 / EC_2 \times 100$$

3.4.4.2 Lipid peroxidation content:

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) present in the leaf tissues. MDA is a product of lipid peroxidation and was measured by thiobarbituric acid (TBA) reaction with minor modifications of the method of Heath and Packer (1968).

Reagents

- (i) 0.1 % Trichloroacetic acid (TCA): One g trichloroacetic acid was dissolved in distilled water and made volume to 1 L.
- (ii) 20 % TCA containing 0.5% TBA: 200 g trichloroacetic acid was dissolved in distilled water. Thereafter, 5 g of thiobarbituric acid was dissolved in it and the final volume was made to 1 L by adding distilled water.

Extraction

300 mg of leaves were homogenized separately with 5 ml of 0.1 % TCA. The

homogenate was centrifuged at 8000 x g for 15 min. The supernatant was then directly used for the assay.

Procedure

To begin the analysis, 1 ml of the supernatant was extracted and then mixed with 4 ml of a 20% solution of TCA that was supplemented with TBA. The mixture was then placed in a water bath shaker at 95⁰C for the time duration of 30 minutes. Immediately following this, it was promptly cooled in an ice bath. Once the cooling process was complete, the mixture underwent centrifugation at a rate of 8000 x g for 10 minutes. At this point, the absorbance of the reaction mixture was read at a wavelength of 532 nm. To account for non-specific absorption, a value was subtracted at a wavelength of 600 nm. To establish a baseline measurement, a blank was prepared using the same solvent employed during the preparation of the sample. Finally, the concentration of MDA was calculated based on its extinction coefficient of 155 mM⁻¹cm⁻¹ and expressed in μmol lit⁻¹.

3.4.5 Antioxidant defence system:

3.4.5.1 Enzymes (specific activity):

Enzyme Extraction

To extract enzyme samples, each leaf sample (weighing 0.5 g when fresh) was homogenized separately in a potassium phosphate buffer (50 mM) kept at 0-4⁰C. The buffer was also made up of 0.1 mM ethylene diamine tetra acetic acid (EDTA) and 1% polyvinyl pyrrolidone (PVP). After the homogenate was filtered through four layers of cheesecloth, it was then centrifuged at 15000 x g for 20 minutes at a temperature of 4⁰C. The supernatant was then collected and an appropriate volume was diluted for enzyme assays. All enzyme extraction procedures were conducted while maintaining a temperature of 0-4⁰C.

Preparation of Enzyme extraction buffer (50 mM)

Potassium hydrogen phosphate solution (0.1 M) was prepared by dissolving 1.74 g chemical in 100 ml dH₂O. Further, potassium dihydrogen phosphate solution (0.1M) was prepared by dissolving 1.36 g of it in 100 ml dH₂O. Solutions of potassium hydrogen phosphate and potassium dihydrogen phosphate were mixed in a ratio of 84:16 and pH was adjusted to 7.0 with pH meter. Thereafter, 7.42 mg EDTA, 2 g PVP and distilled H₂O were added in 100 ml 0.1 M buffer to make the final volume 200 ml.

3.4.5.1.1 Superoxide dismutase activity:

Superoxide dismutase activity (E.C. 1.15.1.1) was estimated according to the method of Dhindsa *et al.* (1981). The reaction mixture comprised 3.0 ml, which included 50 mM phosphate buffer (1.5 ml, pH 7.0), 200 mM L-methionine (0.2 ml), 1.5 M sodium carbonate (0.1 ml), 2.25 mM nitro blue tetrazolium chloride (NBT, 0.1 ml), 3 mM EDTA (0.1 ml), and dH₂O (1.0 ml). The enzyme extract (100 μl) and 2 mM riboflavin (0.1 ml) were added to the reaction mixture to initiate the reaction. The tube was then placed under 2 x 15 W fluorescent

lamps for 15 minutes. To stop the reaction, the light was turned off, and the tube was covered with black cloth. Tubes that lacked enzymes were used as controls to develop maximum colour. A non-irradiated complete reaction mixture served as a blank. The absorbance was measured at 560 nm, and one unit of the enzyme was defined as the quantity of enzyme that reduced the absorbance reading by 50% compared to the tubes without enzymes. The SOD activity was expressed as Units mg^{-1} protein.

Reagents

1. Potassium phosphate buffer (50 mM): Potassium hydrogen phosphate solution (0.1 M) was prepared by dissolving 1.74 g chemical in 100 ml dH_2O . Further, potassium dihydrogen phosphate solution (0.1 M) was prepared by dissolving 1.36 g chemical in 100 ml dH_2O . Solutions of potassium hydrogen phosphate and potassium dihydrogen phosphate were mixed in a ratio of 61:39 and made up a volume of 200 ml by adding distilled water. Then, the pH was adjusted to 7.0.
2. 200 mM L-Methionine: 298 mg of L-Methionine dissolved in dH_2O and the volume was made to 10 ml.
3. 2.25 mM NBT: 18.4 mg of NBT (nitrobluetetrazolium) dissolved in dH_2O and the volume was made to 10 ml and kept airtight.
4. 2 mM riboflavin: 7.52 mg of riboflavin dissolved in dH_2O and volume was made to 10 ml and stored in amber glass bottles.
5. 1.5 M Na_2CO_3 : 1.59 g of Na_2CO_3 dissolved in dH_2O and volume was made to 10 ml.
6. EDTA (3 mM): 87.675 mg EDTA dissolved in dH_2O and volume was adjusted to 100 ml.

3.4.5.1.2 Catalase activity:

Catalase (E.C. 1.11.1.6) activity was measured by following the decrease in absorbance at 240 nm due to the decomposition of H_2O_2 ($\epsilon = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$) in a reaction mixture containing 2 ml 50 mM phosphate buffer (pH 7.0), 1.0 ml 6.66 mM H_2O_2 and 0.1 ml enzyme extract (pre-diluted five times with dH_2O) as described by Chance and Maehly (1955) for 2 min. CAT activity was expressed as Units mg^{-1} protein.

Reagents

1. Phosphate buffer (50mM): Same as prepared for SOD activity.
2. H_2O_2 (6.66 mM): 13.6 μl of 30 % H_2O_2 was diluted to 20 ml with dH_2O .

3.4.5.1.3 Ascorbate peroxidase (APX) activity:

APX (E.C. 1.11.1.11) activity was measured by following the decrease in absorbance at 290 nm due to ascorbate oxidation ($\epsilon = 2.9 \text{ mM}^{-1} \text{ cm}^{-1}$) in the reaction mixture containing 50 mM phosphate buffer (600 μl , pH 7.0), 0.05 mM ascorbic acid (100 μl), 0.1 mM EDTA (100 μl), 1.0 mM H_2O_2 (100 μl) and enzyme extract (100 μl , pre-diluted five times with dH_2O) as described by Nakano and Asada (1981). APX activity was expressed as Units mg^{-1} protein.

Reagents

1. Extraction buffer (50 mM): Same as prepared for SOD activity.
2. H₂O₂ (1.0 mM): 5.1 µl of 30 % H₂O₂ was diluted to 50 ml with dH₂O.
3. EDTA (0.1 mM): 58.45 mg EDTA dissolved in dH₂O and volume was made to 100 ml. Then diluted 5 ml of this stock to 100 ml with dH₂O.
4. Ascorbic acid (0.05 mM): 44 mg of ascorbic acid was diluted to 100 ml with dH₂O. Then diluted 2 ml of this stock to 100 ml with dH₂O.

3.4.5.2 Metabolites

3.4.5.2.1 Ascorbate (oxidized and reduced):

Ascorbate (AsA) content was determined with a modification of the procedure of Takahama and Oniki (1992). Fresh leaf (500 mg) samples were ground separately in 1.5 ml of 2 % (w/v) metaphosphoric acid containing 1 mM EDTA. After centrifugation at 12000 x g at 4⁰C for 5 min, 500 µl of the supernatant was added to 250 µl of 10 % (w/v) sodium citrate. 100 µl of this mixture and 875 µl of 0.2 mM KH₂PO₄ buffer (pH 7.0) and 0.25U of ascorbate oxidase (100 µl) were mixed in cuvette. The absorbance was recorded against buffer blank at 265 nm until no further decrease was observed (3-4 min). Dehydroascorbate (DHA) was reduced by 2 mM dithiothreitol (DTT) and the ascorbate content was calculated by using an extinction coefficient of 15 mM⁻¹ cm⁻¹. The concentration of DHA (ascorbate oxidized) was calculated by the difference between total ascorbate and ascorbate reduced.

Reagents

1. Metaphosphoric acid (2 %) containing 1 mM EDTA: 2 g metaphosphoric acid dissolved in dH₂O. Then 29.22 mg EDTA was added and the volume was made to 100 ml with dH₂O.
2. Sodium citrate (10 %): 10 g sodium citrate diluted to 100 ml with dH₂O.
3. Potassium dihydrogen phosphate (KH₂PO₄) buffer (0.2 mM): 2.72 mg KH₂PO₄ diluted to 100 ml with dH₂O and pH was adjusted to 7.0.
4. Ascorbate oxidase (0.25 U): 25 µl ascorbate oxidase diluted to 10 ml with dH₂O (1 µl of ascorbate oxidase = 1 unit).
5. Dithiothreitol (2mM): 30.85 mg DTT diluted to 100 ml with dH₂O.

3.4.5.2.1 Glutathione (oxidized and reduced):

Fresh leaf (500 mg) samples were homogenized separately in 5 ml of 5 % TCA. After centrifugation at 12000 x g at 4⁰C for 10 min, the supernatant was collected. Total glutathione (GSSG + GSH) was determined by adding 1.75 ml of 0.1 M sodium phosphate buffer (pH 7.5), 0.1 ml of 2 mM NADPH, 0.1 ml of 5 mM DTNB and 0.2 ml of TCA extract. Added one unit of standard glutathione reductase, mixed well, incubated for 10 min and recorded absorbance at 412 nm against reagent blank. For estimation of oxidized glutathione, to 1.0 ml of TCA extract added 0.95 ml of potassium phosphate buffer (0.5 M, pH 7.5) and 50

μl of 4-vinylpyridine. The contents were allowed to stand for 1 h to remove/degrade GSH. To 0.1 ml of vinylpyridine treated extract (which was equivalent to 0.05 ml of original TCA extract) added 1.7 ml of 0.1 M sodium phosphate buffer (pH 7.5), 0.1 ml of 2 mM NADPH and 0.1 ml of DTNB. The reaction was started by adding one unit of standard glutathione reductase (GR) enzyme, incubated for 10 min and absorbance was recorded at 412 nm against reagent blank. Total and oxidized glutathione were calculated by using the equation $y = 0.0014x$ derived from the standard curve of reduced glutathione (100 to 1000 μM). The absorbance of standard GSH was recorded at 412 nm against a buffer blank. The concentration of reduced glutathione (GSH) was calculated by subtracting the concentration of GSSG from that of the total glutathione (Smith *et al.*, 1985).

Reagents

1. Potassium phosphate buffer (0.5 M): Potassium hydrogen phosphate solution (0.5 M) was prepared by dissolving 8.71 g chemical in 100 ml dH_2O . Further, potassium dihydrogen phosphate solution (0.5 M) was prepared by dissolving 6.81 g chemical in 100 ml dH_2O . Solutions of potassium hydrogen phosphate and potassium dihydrogen phosphate were mixed in a ratio of 61:39 and pH was adjusted to 7.0.
2. Sodium phosphate buffer (0.1 M): Sodium hydrogen phosphate solution (1 M) was prepared by dissolving 14.20g chemical in 100 ml dH_2O . Further, sodium dihydrogen phosphate solution (1 M) was prepared by dissolving 12g chemical in 100 ml dH_2O . Solutions of sodium hydrogen phosphate and sodium dihydrogen phosphate were mixed in the ratio of 57.7:42.3 and diluted to 1 L with distilled water and pH adjusted to 7.5.
3. NADPH (2 mM): 166.7 mg NADPH was diluted to 100 ml with dH_2O .
4. 5, 5'-dithiobis-2-nitrobenzoid acid (5 mM): 198.2 mg DTNB was diluted to 100 ml with 0.1 M sodium or potassium phosphate buffer.
5. Glutathione reductase (1 U): 100 μl glutathione reductase diluted to 10 ml with dH_2O (1 μl of glutathione reductase = 1 unit).
6. 4-vinyl pyridine

3.4.6 Yield and yield attributing characters: Following data of yield and its attributing characters were recorded at the time of harvest:

3.4.6.1 Number of primary branches per plant: It was recorded at the time of maturity by counting the number of branches emerging directly from the main stem-bearing siliquae.

3.4.6.2 Number of secondary branches per plant: It was recorded at the time of maturity by counting the number of siliquae-bearing branches emerging from the primary branches.

3.4.6.3 Main shoot length (cm): The length of the main raceme was measured at the time of maturity in centimetres from the point it emerges out from the main stem up to the end point of it.

3.4.6.4 Number of siliqua per plant: Total number of siliquae of randomly selected plants was counted and the average value was calculated. Five siliquae taken for measuring siliqua length were also added to the total number.

3.4.6.5 Number of seeds per siliqua: The number of seeds in five siliquae used for measuring average siliqua length was counted and averaged.

3.4.6.6 1000 seed weight (g): One thousand seeds counted from each plant in each replication were weighed in grams on digital electronic balances.

3.4.6.7 Biological yield (kg/ha): At harvest, the above-ground phytomass of the randomly selected 5 plants was removed and kept in sunlight. After sun drying, the samples were kept in a hot air oven at 80°C for 48 hours for complete drying. Then, the material was weighed and the average was used as biological yield. The biological yield was calculated with the following formula:

$$\text{Biological Yield (kg/ha)} = \text{Biological yield (kg) plot}^{-1} / \text{Area plot}^{-1} \times 10000$$

3.4.6.8 Seed yield (kg/ha): After harvesting and threshing, the seed from 5 randomly selected plants was removed. Then, seeds were weighed and the average was used as seed yield. Seed yield was calculated with the following formula:

$$\text{Seed Yield (kg/ha)} = \text{Seed yield (kg) plot}^{-1} / \text{Area plot}^{-1} \times 10000$$

3.4.6.9 Stover yield (kg/ha): After harvesting and threshing, the seed was removed from 5 randomly selected plants, and then, the remained part of the plant is stover yield. Stover yield was calculated with the following formula:

$$\text{Stover yield (Kg/ha)} = \text{Biological yield (kg/ha)} - \text{Seed yield (kg/ha)}$$

3.4.6.10 Harvest index (%): It denoted the ratio of seed yield to biological yield and was calculated with the following formula:

$$\text{Harvest index (\%)} = \text{Seed yield} / \text{Biological yield} \times 100$$

3.4.7 Soil parameters (%):

3.4.7.1 Soil moisture: For the estimation of soil moisture, soil samples of 75-100 gm from each treatment were collected at a depth of 0-30 cm, 30-60 cm and 60-90 cm by using a soil auger at 30DAS, 60DAS, 90 DAS, 120DAS and at the time of harvesting. Samples were taken as quickly as possible and stored in soil moisture boxes. The fresh weight of the samples was measured, and dried in an oven at 80°C for 72 hours and dry weight was measured. Soil moisture content was calculated according to the procedure suggested by Ge *et al.*, (2012) from the following formula:

$$\text{Soil moisture content (\%)} = [\text{Soil fresh weight} - \text{Soil dry weight} / \text{Soil dry weight}] \times 100$$

The present investigation aimed to study the response of hydrogels and salicylic acid for drought mitigation in *Brassica juncea*. The hydrogels were applied at the time of sowing in the soil while SA was applied through foliar spray at flowering and siliqua formation stages in different combinations. All observations were recorded after the foliar spray of salicylic acid. The results presented in this chapter along with the data are supplemented with different tables under the following headings.

4.1 Growth and reproductive parameters

4.2 Physiological parameters

4.3 Biochemical parameters

4.4 Membrane injury indices

4.5 Antioxidant defence system

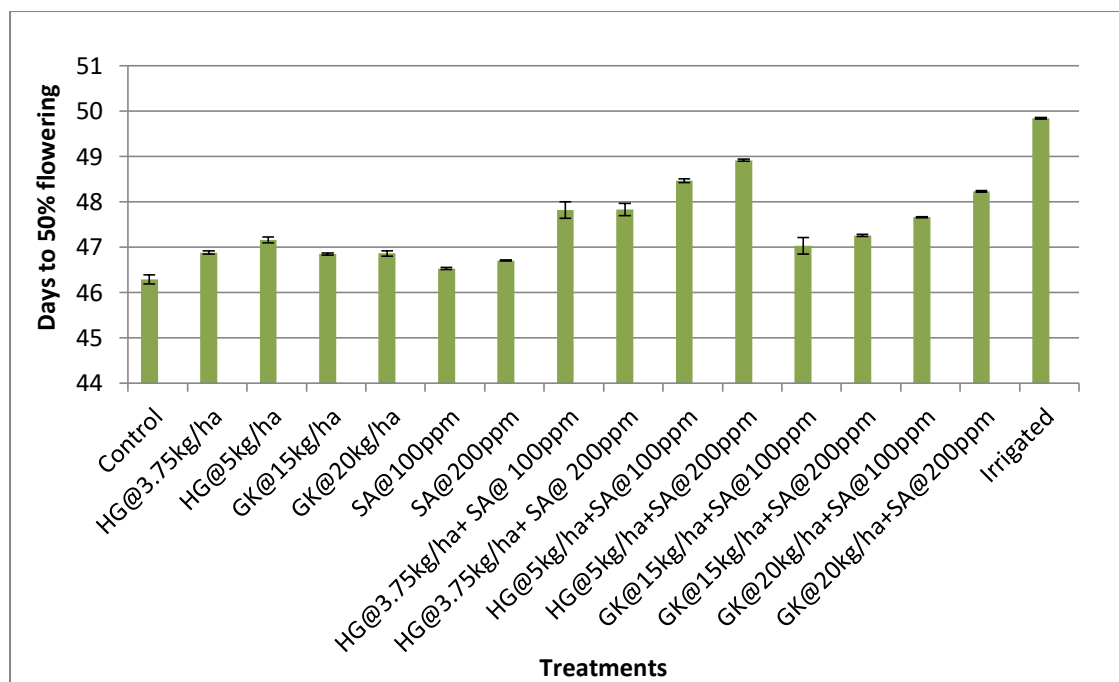
4.6 Yield and yield attributing characters

4.7 Soil parameters

4.1 Growth and reproductive parameters:

4.1.1 Days to 50% flowering

The data presented in Figure 3 illustrates the significant impact of different treatments on the duration to achieve 50% flowering. The application of hydrogel at the rate of 5kg/ha, followed by two foliar sprays of 200 ppm salicylic acid during the flowering and siliqua formation stages, led to a significant delay of 2.63 days in 50 % flowering. This treatment was followed by the application of hydrogel at the same rate along with two foliar sprays of 100 ppm salicylic acid during the flowering and siliqua formation stages. The control (rainfed) conditions had the shortest duration to 50 % flowering at 46.29 days, while the longest duration was observed under irrigated conditions at 49.85 days. In terms of % reduction, days to 50 % flowering reduced by 7.6 % under the rainfed condition when compared with the irrigated condition. The application of hydrogel at a rate of 5kg/ha with 200 ppm salicylic acid and 100 ppm salicylic acid delayed the duration until 50 % flowering by 5.6 % and 4.7 %, respectively as compared to control conditions. The results showed that GK treatments also delayed the 50% flowering duration when compared with the control conditions but showed early flowering when compared with irrigated and hydrogel treatments.

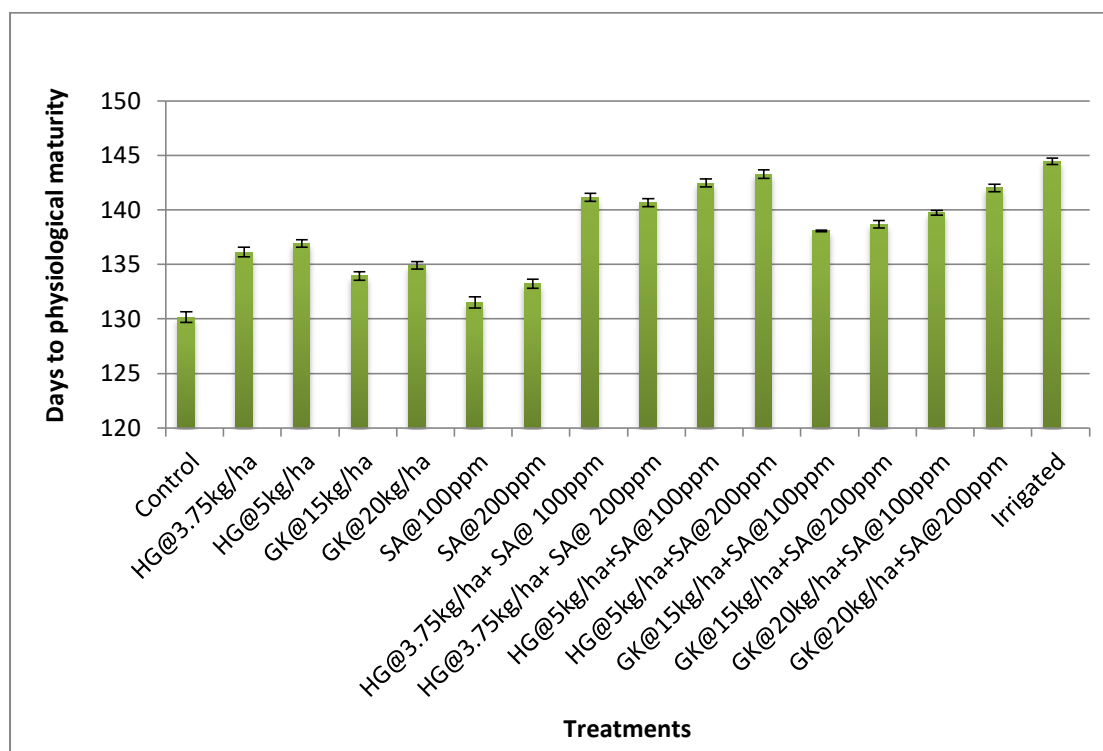


**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

Figure 3: Effect of salicylic acid and hydrogel treatments on days to 50% flowering of Indian mustard variety RH 725 under rainfed conditions (Values are means of two *Rabi* seasons (2019-20 and 2020-21) and vertical bars indicate standard error (\pm))

4.1.2 Days to physiological maturity

The number of days required for physiological maturity was notably impacted by rainfed conditions. In contrast, Brassica plants that were grown under irrigated conditions exhibited standard maturity timings, whereas, under rainfed conditions, they showed signs of maturing earlier. Furthermore, the use of hydrogel and salicylic acid also resulted in early maturity, surpassing even the irrigated condition, as depicted in Figure 5. The results depicted in Figure 4 indicated the significant impact of different treatments. The most effective treatment was HG@5kg/ha+SA (200 ppm) followed by HG@5kg/ha+SA (100 ppm), in terms of increasing days taken for physiological maturity as compared to rainfed plants. Among all the treatments, the maximum number of days taken for maturity was observed in irrigated conditions (144.46 days), while the minimum was in rainfed conditions (130.15 days). The control (rainfed) plants showed the greatest reduction in time to maturity, with a decrease of 10.2 %, which was further improved by 9.3 % and 8.7 % with the application of hydrogel at a rate of 5kg/ha with 200 ppm SA and 100 ppm SA, respectively as compared to rainfed plants. The results showed that GK treatments also delayed the maturity time when compared with the control conditions but showed early maturity when compared with irrigated and hydrogel treatments.

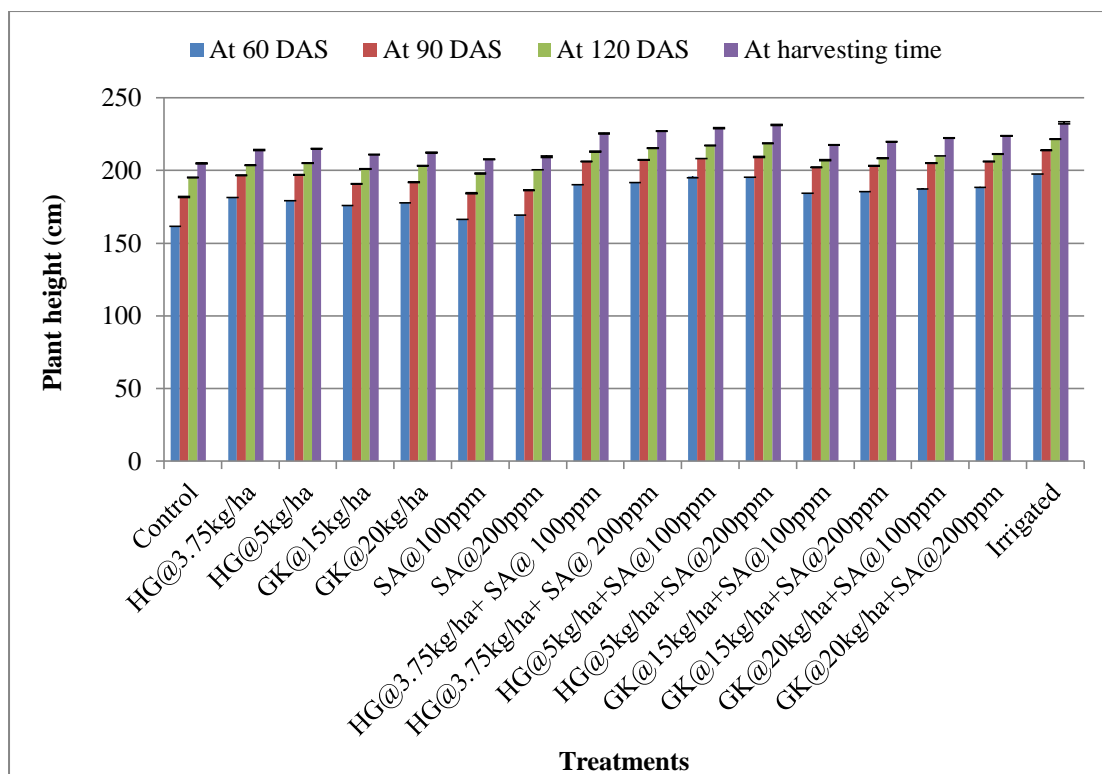


**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

Figure 4: Effect of salicylic acid and hydrogel treatments on days to physiological maturity of Indian mustard variety RH 725 under rainfed conditions (Values are means of two *Rabiseasons* (2019-20 and 2020-21) and vertical bars indicate standard error (\pm))

4.1.3 Plant height (cm)

Throughout the various stages of observation, significant disparities in plant height were noted among all treatments (Figure 5). When hydrogel was applied in conjunction with salicylic acid as foliar sprays, a marked increase in plant height was observed in comparison to the control (rainfed) treatment. At the harvesting stage, the control (rainfed) conditions recorded the greatest decrease in plant height (13.6 %), while the least decrease was seen in the hydrogel@5kg/ha+SA (200 ppm) treatment (12.8 %), followed by hydrogel@5kg/ha+SA (100 ppm) (11.9 %), when compared to irrigated conditions. The maximum plant height among treatments was observed in the irrigated treatment (232.69 cm), followed by hydrogel@5kg/ha+SA (200 ppm) (231.32 cm) and hydrogel@5kg/ha+SA (100 ppm) (229.26 cm), while the minimum plant height was recorded in the control (rainfed) treatment (204.80 cm). The results showed that GK treatments also increase the plant height when compared with the control conditions but less than irrigated and hydrogel treatments.



**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

Figure 5: Effect of salicylic acid and hydrogel treatments on plant height (cm) of Indian mustard variety RH 725 under rainfed conditions (Values are means of two Rabi seasons (2019-20 and 2020-21) and vertical bars indicate standard error (\pm))

4.2 Physiological parameters:

4.2.1 Photosynthetic rate

The data presented in Table 1 showed that, during the flowering stage, the photosynthetic rate was significantly reduced with rainfed and all other treatments as compared to the irrigated plants. The rate of photosynthesis under irrigated conditions was significantly higher than that under rainfed conditions. The application of hydrogel at a rate of 5kg/ha, along with two foliar sprays of salicylic acid at the flowering and siliqua formation stages, resulted in a significant increase in photosynthetic rates compared to the control (rainfed) conditions. At the siliqua formation stage, similar results were observed in the flowering stage, but a decrease in values was noted at the siliqua formation stage. At this stage, the control (rainfed) conditions showed the maximum reduction in photosynthetic rate (51.4 %), while the hydrogel with 5kg/ha with 200ppm salicylic acid treatment displayed the minimum reduction (9.4 %) as compared to irrigated conditions. This was followed by treatments of hydrogel@5kg/ha+ SA (100ppm) (13.9 %) and hydrogel@3.75kg/ha + SA (200 ppm) (18.7 %), as compared to irrigated conditions. The highest photosynthetic rate was recorded under the irrigated treatment (18.06), followed by the hydrogel@5kg/ha + 200 ppm salicylic acid treatment (16.94). The results showed that GK treatments also improved the photosynthetic rate when compared with the control conditions but it does not improve the photosynthetic rate like irrigated and hydrogel treatments.

Table 1: Effect of salicylic acid and hydrogel treatments on photosynthetic rate ($\mu\text{molCO}_2\text{m}^{-1}\text{s}^{-1}$) of Indian mustard variety RH 725 under rainfed conditions

Treatments		Photosynthetic rate ($\mu\text{molCO}_2\text{m}^{-1}\text{s}^{-1}$)					
		At flowering stage			At siliqua formation stage		
		2019-20	2020-21	Mean	2019-20	2020-21	Mean
T ₁	Control (RF)	14.46 ± 0.28	13.83 ± 0.08	14.14 ^l	12.42 ± 0.21	11.43 ± 0.20	11.92 ⁱ
T ₂	HG@3.75kg/ha	15.55 ± 0.33	14.74 ± 0.08	15.14 ^{ij}	13.19 ± 0.11	12.53 ± 0.20	12.86 ^{gh}
T ₃	HG@5kg/ha	16.32 ± 0.32	15.55 ± 0.08	15.93 ^h	13.72 ± 0.09	13.12 ± 0.16	13.42 ^{fg}
T ₄	GK@15kg/ha	15.01 ± 0.04	14.80 ± 0.13	14.91 ^{ijk}	13.19 ± 0.08	12.32 ± 0.06	12.75 ^{gh}
T ₅	GK@20kg/ha	15.43 ± 0.29	15.09 ± 0.12	15.26 ⁱ	13.49 ± 0.07	12.49 ± 0.07	12.99 ^{fgh}
T ₆	SA@100ppm	14.95 ± 0.02	14.10 ± 0.08	14.52 ^{kl}	12.85 ± 0.06	12.28 ± 0.23	12.56 ^{hi}
T ₇	SA@200ppm	15.21 ± 0.14	14.26 ± 0.15	14.74 ^k	13.06 ± 0.04	12.63 ± 0.29	12.84 ^{gh}
T ₈	HG@3.75kg/ha+ SA@100ppm	18.09 ± 0.07	17.87 ± 0.06	17.98 ^d	15.88 ± 0.05	14.82 ± 0.06	15.35 ^d
T ₉	HG@3.75kg/ha+ SA@200ppm	18.71 ± 0.14	18.15 ± 0.08	18.43 ^d	16.33 ± 0.17	15.33 ± 0.16	15.83 ^{cd}
T ₁₀	HG@5kg/ha+ SA@100ppm	19.20 ± 0.15	18.72 ± 0.08	18.96 ^c	16.89 ± 0.06	15.92 ± 0.07	16.40 ^{bc}
T ₁₁	HG@5kg/ha+ SA@200ppm	19.93 ± 0.10	19.60 ± 0.27	19.76 ^b	17.42 ± 0.06	16.46 ± 0.05	16.94 ^b
T ₁₂	GK@15kg/ha+ SA@100ppm	16.46 ± 0.11	15.72 ± 0.09	16.09 ^{gh}	13.77 ± 0.04	13.64 ± 0.07	13.70 ^{ef}
T ₁₃	GK@15kg/ha+ SA@200ppm	16.93 ± 0.03	16.16 ± 0.13	16.54 ^{fg}	14.24 ± 0.06	14.24 ± 0.12	14.24 ^e
T ₁₄	GK@20kg/ha+ SA@100ppm	17.29 ± 0.10	16.63 ± 0.09	16.96 ^{ef}	14.82 ± 0.07	15.26 ± 0.12	15.04 ^d
T ₁₅	GK@20kg/ha+ SA@200ppm	17.95 ± 0.03	16.96 ± 0.03	17.45 ^e	15.21 ± 0.08	15.56 ± 0.15	15.38 ^d
T ₁₆	Irrigated (IR)	20.76 ± 0.17	21.08 ± 0.56	20.92 ^a	17.92 ± 0.02	18.20 ± 0.06	18.06 ^a
Mean		17.01	16.45	16.73	14.65	14.14	14.39
SE(m)		0.18	0.19	0.17	0.09	0.14	0.26
CD at 5% level of significance		0.54	0.55	0.51	0.27	0.42	0.80
CV (%)		0.18	0.20	0.14	0.11	0.18	0.26

**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

**Treatments with the same letters are not significantly different

4.2.2 Transpiration rate

The data presented in Table 2 revealed that, during the flowering stage, the transpiration rate was significantly reduced under rainfed conditions, however irrigation of crops showed a significantly higher transpiration rate. To increase the transpiration rate, the application of hydrogel@5kg/ha in tandem with two foliar sprays of 200ppm salicylic acid at the siliqua formation and flowering stages proved to be effective. This treatment yielded significant results when compared to the control treatment. While similar results were observed at the siliqua formation stage as were observed during the flowering stage, the values were decreased. At the siliqua formation stage, the control (rainfed) treatment experienced the greatest reduction in transpiration rate (88.1%), while the hydrogel@5kg/ha+SA (200ppm) treatment showed the least reduction (10%), followed by hydrogel@5kg/ha+SA (100ppm) (18.9%) and hydrogel@3.75kg/ha+SA (200ppm) (29.4%) when compared to the irrigated condition. The irrigated treatment had the highest transpiration rate (3.032), followed by hydrogel@5kg/ha+SA (200ppm) (2.955) and hydrogel@5kg/ha+SA (100ppm) (2.818). The data presented in Table 2 revealed that, GK treatments also improved the transpiration rate when compared with the control conditions.

4.2.3 Stomatal Conductance

The data presented in Table 3 depicts that, during the flowering stage, the stomatal conductance was significantly lesser under rainfed conditions as compared to other treatments of HG and SA, in combination and IR conditions. Stomatal conductance was found to be noticeably higher when plants were irrigated as opposed to when they were rainfed. When hydrogel was applied at a rate of 5kg/ha along with two foliar sprays of salicylic acid at the flowering and siliqua formation stages, there was a significant increase in stomatal conductance as compared to the rainfed condition. Similar results were observed during the siliqua formation stage compared to the flowering stage, albeit with lower values. At the siliqua formation stage, the maximum reduction in stomatal conductance was noted in the rainfed condition (86 %), while the hydrogel (5kg/ha) with 200 ppm salicylic acid exhibited the minimum reduction (9.3 %) as compared to irrigated conditions. This was followed by hydrogel@5kg/ha+ SA (100 ppm) (15.2 %) and gond katira @(20kg/ha)+ SA (200 ppm) (20.1 %).

The highest stomatal conductance was observed in the irrigated plants (0.383), followed by hydrogel@5kg/ha+ SA (200 ppm) (0.386) and gond katira @(20kg/ha)+ SA (200 ppm) (0.355).

Table 2: Effect of salicylic acid and hydrogel treatments on transpiration rate (mmol H₂O m⁻² s⁻¹) of Indian mustard variety RH 725 under rainfed conditions

Treatments		Transpiration rate (mmol H ₂ O m ⁻² s ⁻¹)					
		At flowering stage			At siliqua formation stage		
		2019-20	2020-21	Mean	2019-20	2020-21	Mean
T ₁	Control(RF)	1.830 ± 0.017	1.820 ± 0.017	1.825 ⁱ	1.553 ± 0.029	1.437 ± 0.029	1.495 ⁱ
T ₂	HG@3.75kg/ha	1.940 ± 0.015	2.080 ± 0.021	2.010 ^{ghi}	1.913 ± 0.018	1.767 ± 0.015	1.840 ^{gh}
T ₃	HG@5kg/ha	1.987 ± 0.009	2.227 ± 0.019	2.107 ^{gh}	1.950 ± 0.006	1.820 ± 0.021	1.885 ^{gh}
T ₄	GK@15kg/ha	1.900 ± 0.012	1.978 ± 0.009	1.939 ^{hi}	1.797 ± 0.015	1.733 ± 0.019	1.765 ^h
T ₅	GK@20kg/ha	1.927 ± 0.003	2.012 ± 0.018	1.969 ^{hi}	1.840 ± 0.006	1.747 ± 0.02	1.795 ^{gh}
T ₆	SA@100ppm	1.857 ± 0.009	1.833 ± 0.010	1.845 ⁱ	1.737 ± 0.009	1.653 ± 0.02	1.695 ^{hi}
T ₇	SA@200ppm	1.883 ± 0.009	1.878 ± 0.012	1.880 ⁱ	1.777 ± 0.012	1.723 ± 0.012	1.750 ^{hi}
T ₈	HG@3.75kg/ha+ SA@100ppm	2.670 ± 0.021	2.725 ± 0.015	2.697 ^d	2.217 ± 0.012	2.577 ± 0.024	2.397 ^{de}
T ₉	HG@3.75kg/ha+ SA@200ppm	2.520 ± 0.015	2.738 ± 0.015	2.629 ^d	2.563 ± 0.019	2.853 ± 0.012	2.708 ^{bc}
T ₁₀	HG@5kg/ha+ SA@100ppm	3.053 ± 0.012	3.173 ± 0.022	3.113 ^{bc}	2.710 ± 0.015	2.927 ± 0.012	2.818 ^{ab}
T ₁₁	HG@5kg/ha+ SA@200ppm	3.223 ± 0.015	3.316 ± 0.015	3.269 ^{ab}	2.823 ± 0.012	3.087 ± 0.032	2.955 ^{ab}
T ₁₂	GK@15kg/ha+ SA@100ppm	2.047 ± 0.015	2.365 ± 0.023	2.206 ^{fg}	2.013 ± 0.015	2.083 ± 0.038	2.048 ^{fg}
T ₁₃	GK@15kg/ha+ SA@200ppm	2.143 ± 0.012	2.558 ± 0.020	2.350 ^{ef}	2.087 ± 0.015	2.237 ± 0.041	2.162 ^{ef}
T ₁₄	GK@20kg/ha+ SA@100ppm	2.337 ± 0.019	2.648 ± 0.015	2.492 ^{de}	2.143 ± 0.012	2.327 ± 0.018	2.235 ^{def}
T ₁₅	GK@20kg/ha+ SA@200ppm	2.930 ± 0.015	2.885 ± 0.017	2.907 ^c	2.333 ± 0.018	2.563 ± 0.019	2.448 ^{cd}
T ₁₆	Irrigated(IR)	3.337 ± 0.015	3.562 ± 0.030	3.449 ^a	2.947 ± 0.023	3.117 ± 0.029	3.032 ^a
Mean		2.349	2.487	2.418	2.150	2.228	2.189
SE(m)		0.012	0.016	0.068	0.013	0.022	0.087
CD at 5% level of significance		0.035	0.046	0.206	0.038	0.065	0.261
CV (%)		0.895	0.110	0.403	0.105	0.174	0.559

**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

**Treatments with same letters are not significantly different

Table 3: Effect of salicylic acid and hydrogel treatments on stomatal conductance ($\text{mmolH}_2\text{Om}^{-2}\text{s}^{-1}$) of Indian mustard variety RH 725 under rainfed conditions

Treatments		Stomatal conductance ($\text{mmol H}_2\text{Om}^{-2}\text{s}^{-1}$)					
		At flowering stage			At siliqua formation stage		
		2019-20	2020-21	Mean	2019-20	2020-21	Mean
T ₁	Control(RF)	0.283 ± 0.003	0.210 ± 0.006	0.246 ⁱ	0.197 ± 0.009	0.177 ± 0.009	0.187 ^g
T ₂	HG@3.75kg/ha	0.330 ± 0.006	0.317 ± 0.009	0.323 ^{gh}	0.250 ± 0.012	0.207 ± 0.012	0.228 ^g
T ₃	HG@5kg/ha	0.357 ± 0.007	0.333 ± 0.009	0.345 ^{fg}	0.270 ± 0.006	0.287 ± 0.009	0.278 ^{ef}
T ₄	GK@15kg/ha	0.307 ± 0.003	0.247 ± 0.003	0.277 ⁱ	0.220 ± 0.006	0.196 ± 0.003	0.208 ^g
T ₅	GK@20kg/ha	0.313 ± 0.007	0.265 ± 0.003	0.289 ^{hi}	0.240 ± 0.006	0.220 ± 0.006	0.230 ^{fg}
T ₆	SA@100ppm	0.297 ± 0.003	0.223 ± 0.003	0.260 ⁱ	0.207 ± 0.007	0.180 ± 0.026	0.193 ^g
T ₇	SA@200ppm	0.303 ± 0.003	0.233 ± 0.003	0.268 ⁱ	0.220 ± 0.006	0.190 ± 0.006	0.205 ^g
T ₈	HG@3.75kg/ha+SA@100ppm	0.403 ± 0.007	0.383 ± 0.003	0.393 ^{bcd}	0.299 ± 0.006	0.337 ± 0.009	0.318 ^{cde}
T ₉	HG@3.75kg/ha+SA@200ppm	0.400 ± 0.006	0.410 ± 0.006	0.405 ^{bcd}	0.310 ± 0.006	0.347 ± 0.003	0.328 ^{bcd}
T ₁₀	HG@5kg/ha+ SA@100ppm	0.433 ± 0.003	0.413 ± 0.003	0.423 ^{abc}	0.333 ± 0.003	0.377 ± 0.003	0.355 ^{abc}
T ₁₁	HG@5kg/ha+ SA@200ppm	0.450 ± 0.006	0.423 ± 0.003	0.436 ^{ab}	0.343 ± 0.003	0.393 ± 0.003	0.368 ^{ab}
T ₁₂	GK@15kg/ha+ SA@100ppm	0.350 ± 0.006	0.350 ± 0.006	0.350 ^{efg}	0.270 ± 0.006	0.293 ± 0.012	0.281 ^{de}
T ₁₃	GK@15kg/ha+ SA@200ppm	0.373 ± 0.003	0.367 ± 0.007	0.370 ^{def}	0.277 ± 0.003	0.303 ± 0.009	0.290 ^{de}
T ₁₄	GK@20kg/ha+ SA@100ppm	0.383 ± 0.003	0.397 ± 0.009	0.390 ^{cde}	0.290 ± 0.006	0.333 ± 0.006	0.310 ^{cde}
T ₁₅	GK@20kg/ha+ SA@200ppm	0.420 ± 0.006	0.400 ± 0.006	0.410 ^{bcd}	0.323 ± 0.003	0.366 ± 0.006	0.341 ^{abc}
T ₁₆	Irrigated(IR)	0.463± 0.003	0.457 ± 0.012	0.460 ^a	0.360 ± 0.006	0.407 ± 0.003	0.383 ^a
Mean		0.366	0.339	0.352	0.275	0.287	0.281
SE(m)		0.005	0.006	Inf	0.006	0.010	Inf
CD at 5% level of significance		0.015	0.018	0.044	0.017	0.028	0.050
CV (%)		0.236	0.310	0.583	0.369	0.330	0.830

**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

**Treatments with the same letters are not significantly different

4.2.4 Relative water content (%)

Observation of data revealed that at the flowering stage, RWC was gradually diminished in the rainfed condition in comparison to all other treatments (Table 4). However, considerable improvement in RWC was observed with the application of hydrogel@5kg/ha along with two foliar sprays of 200ppm salicylic acid at both the flowering and siliqua formation stages. This increase in RWC was also seen in hydrogel@3.75kg/ha with two foliar sprays of 200 ppm salicylic acid at both the flowering and siliqua formation stages, as compared to rainfed treatments. Among all treatments, the irrigated treatment exhibited the highest RWC at both sampling stages.

The RWC levels at the siliqua formation stage followed a similar trend as at the flowering stage but with lower values ranging from 77.84 to 92.85 % and 70.86 to 87.55 % respectively. At the siliqua formation stage, the control condition (rainfed) showed a maximum reduction in RWC by 27.4 %, which was later improved by 24.7 % and 23 % with the application of hydrogel@5kg/ha+SA(200 ppm) and hydrogel@3.75kg/ha+SA (200 ppm), respectively as compared to irrigated conditions. The results showed that GK treatments also improved RWC when compared with the control conditions but showed less RWC when compared with irrigated and hydrogel treatments.

4.2.5 Water Potential (Ψ_w)

Data depicted in Table 5 revealed that at the flowering stage, the water potential of the mustard plant was gradually reduced in rainfed conditions in comparison to all other treatments. The application of hydrogel at a rate of 5kg/ha, coupled with two foliar sprays of 200ppm salicylic acid during the flowering and siliqua formation stages, resulted in a significant increase in Ψ_w .

The same effect was observed with the hydrogel at 5kg/ha with two foliar sprays of 100 ppm salicylic acid during the same growth stages, compared to corresponding treatments. The highest Ψ_w was noted in the irrigated treatment at both sampling stages, surpassing all other treatments. The Ψ_w levels observed during the siliqua formation stage followed a similar trend as at the flowering stage, although with decreased (more negative) levels. The values ranged from 2.090 to 1.593 and 2.168 to 1.610 -MPa at flowering and siliqua formation stages, respectively.

At the siliqua formation stage, the control (rainfed) condition induced the maximum reduction in Ψ_w by 25.7 %, while the application of hydrogel at 5kg/ha+SA (200 ppm) and hydrogel at 5kg/ha+SA (100 ppm) alleviated the reduction in Ψ_w by 25.4 % and 23.9 %, respectively. The results showed that GK treatments also improved the Ψ_w when compared with the control conditions.

4.2.6 Osmotic potential (Ψ_s)

About data presented in Table 6 showed that at the flowering stage, the osmotic potential of the mustard plant was gradually reduced in rainfed conditions in comparison to

Table 4: Effect of salicylic acid and hydrogel treatments on relative water content (%) of leaves in Indian mustard variety RH 725 under rainfed conditions

Treatments		Relative water content (%)					
		At flowering stage			At siliqua formation stage		
		2019-20	2020-21	Mean	2019-20	2020-21	Mean
T ₁	Control(RF)	76.99 ± 0.06	78.69 ± 0.12	77.84 ^l	71.52 ± 0.23	70.21 ± 0.09	70.86 ^m
T ₂	HG@3.75kg/ha	83.74 ± 0.39	85.09 ± 0.12	84.42 ^h	76.25 ± 0.08	77.43 ± 0.05	76.84 ^j
T ₃	HG@5kg/ha	84.04 ± 0.05	85.76 ± 0.07	84.90 ^{gh}	77.21 ± 0.12	78.52 ± 0.16	77.86 ^{ij}
T ₄	GK@15kg/ha	80.03 ± 0.03	81.35 ± 0.09	80.69 ^{ij}	73.55 ± 0.21	74.44 ± 0.15	73.99 ^k
T ₅	GK@20kg/ha	81.35 ± 0.24	82.49 ± 0.13	81.92 ⁱ	74.42 ± 0.14	74.35 ± 0.69	74.38 ^k
T ₆	SA@100ppm	78.72 ± 0.10	79.11 ± 0.05	78.91 ^{kl}	72.50 ± 0.09	71.28 ± 0.09	71.89 ^{lm}
T ₇	SA@200ppm	79.18 ± 0.17	80.37 ± 0.07	79.77 ^{jk}	73.43 ± 0.16	72.71 ± 0.15	73.07 ^{kl}
T ₈	HG@3.75kg/ha+SA@100ppm	88.44 ± 0.21	87.39 ± 0.12	87.91 ^{de}	82.40 ± 0.12	83.75 ± 0.08	83.07 ^{de}
T ₉	HG@3.75kg/ha+ SA@200ppm	90.45 ± 0.16	89.55 ± 0.09	90.00 ^{bc}	84.36 ± 0.12	85.46 ± 0.10	84.91 ^{bc}
T ₁₀	HG@5kg/ha+ SA@100ppm	89.54 ± 0.23	88.26 ± 0.11	88.90 ^{cd}	83.33 ± 0.19	84.69 ± 0.11	84.01 ^{cd}
T ₁₁	HG@5kg/ha+ SA@200ppm	91.65 ± 0.12	90.35 ± 0.14	91.65 ^b	85.46 ± 0.12	86.37 ± 0.19	85.91 ^b
T ₁₂	GK@15kg/ha+ SA@100ppm	84.18 ± 0.05	85.92 ± 0.04	85.05 ^{gh}	78.44 ± 0.09	79.44 ± 0.21	78.94 ^{hi}
T ₁₃	GK@15kg/ha+ SA@200ppm	85.35 ± 0.10	86.17 ± 0.04	85.76 ^{fgh}	79.27 ± 0.14	80.44 ± 0.05	79.85 ^{gh}
T ₁₄	GK@20kg/ha+ SA@100ppm	86.24 ± 0.13	86.83 ± 0.03	86.54 ^{efg}	80.74 ± 0.13	81.56 ± 0.19	81.15 ^{fg}
T ₁₅	GK@20kg/ha+ SA@200ppm	87.41 ± 0.22	87.14 ± 0.04	87.27 ^{def}	81.29 ± 0.081	82.64 ± 0.15	81.97 ^{ef}
T ₁₆	Irrigated(IR)	93.31 ± 0.12	92.38 ± 0.10	92.85 ^a	87.51 ± 0.20	87.60 ± 0.20	87.55 ^a
Mean		84.49	84.96	85.27	79.43	78.85	79.14
SE(m)		0.17	0.09	0.57	0.15	0.22	0.46
CD at 5% level of significance		0.51	0.27	1.74	0.44	0.64	1.39
CV (%)		0.41	0.21	0.10	0.38	0.55	0.94

**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

**Treatments with the same letters are not significantly different

Table 5: Effect of salicylic acid and hydrogel treatments on water potential (-MPa) of Indian mustard variety RH 725 under rainfed conditions

Treatments		Water potential (-MPa)					
		At flowering stage			At siliqua formation stage		
		2019-20	2020-21	Mean	2019-20	2020-21	Mean
T ₁	Control(RF)	2.080 ± 0.006	2.100 ± 0.006	2.090 ^l	2.160 ± 0.006	2.177 ± 0.003	2.168 ^l
T ₂	HG@3.75kg/ha	1.860 ± 0.006	1.877 ± 0.003	1.868 ⁱ	1.910 ± 0.006	1.923 ± 0.003	1.916 ^h
T ₃	HG@5kg/ha	1.800 ± 0.006	1.817 ± 0.003	1.808 ^h	1.843 ± 0.003	1.857 ± 0.003	1.850 ^g
T ₄	GK@15kg/ha	1.930 ± 0.006	1.953 ± 0.003	1.941 ^{jk}	1.963 ± 0.003	1.977 ± 0.003	1.970 ⁱ
T ₅	GK@20kg/ha	1.897 ± 0.009	1.917 ± 0.003	1.907 ^{ij}	1.923 ± 0.003	1.943 ± 0.003	1.933 ^h
T ₆	SA@100ppm	1.903 ± 0.052	2.033 ± 0.033	1.968 ^k	2.013 ± 0.003	2.033 ± 0.003	2.023 ^k
T ₇	SA@200ppm	1.943 ± 0.003	1.960 ± 0.006	1.951 ^k	1.987 ± 0.003	2.007 ± 0.003	1.997 ^j
T ₈	HG@3.75kg/ha+ SA@100ppm	1.660 ± 0.006	1.677 ± 0.003	1.668 ^{cd}	1.677 ± 0.003	1.700 ± 0.006	1.688 ^{cd}
T ₉	HG@3.75kg/ha+ SA@200ppm	1.637 ± 0.003	1.663 ± 0.335	1.650 ^{bcd}	1.667 ± 0.003	1.687 ± 0.003	1.677 ^c
T ₁₀	HG@5kg/ha+ SA@100ppm	1.617 ± 0.003	1.643 ± 0.009	1.630 ^{abc}	1.637 ± 0.003	1.660 ± 0.009	1.648 ^b
T ₁₁	HG@5kg/ha+ SA@200ppm	1.597 ± 0.003	1.623 ± 0.003	1.610 ^{ab}	1.607 ± 0.003	1.627 ± 0.003	1.617 ^a
T ₁₂	GK@15kg/ha+ SA@100ppm	1.777 ± 0.003	1.803 ± 0.006	1.790 ^{gh}	1.837 ± 0.003	1.863 ± 0.003	1.850 ^g
T ₁₃	GK@15kg/ha+ SA@200ppm	1.747 ± 0.003	1.767 ± 0.006	1.757 ^{fg}	1.803 ± 0.003	1.820 ± 0.009	1.811 ^f
T ₁₄	GK@20kg/ha+ SA@100ppm	1.707 ± 0.003	1.727 ± 0.003	1.717 ^{ef}	1.743 ± 0.003	1.703 ± 0.006	1.723 ^e
T ₁₅	GK@20kg/ha+ SA@200ppm	1.687 ± 0.003	1.693 ± 0.003	1.690 ^{de}	1.697 ± 0.003	1.720 ± 0.003	1.708 ^{de}
T ₁₆	Irrigated (IR)	1.583 ± 0.003	1.603 ± 0.003	1.593 ^a	1.600 ± 0.006	1.620 ± 0.006	1.610 ^a
Mean		1.789	1.803	1.790	1.816	1.832	1.824
SE(m)		0.014	0.009	0.014	0.004	0.004	0.008
CD at 5% level of significance		0.04	0.026	0.042	0.011	0.012	0.023
CV (%)		0.135	0.872	0.110	0.369	0.377	0.591

**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

**Treatments with the same letters are not significantly different

Table 6: Effect of salicylic acid and hydrogel treatments on osmotic potential (-MPa) of Indian mustard variety RH 725 under rainfed conditions

Osmotic potential (-MPa)							
Treatments		At flowering stage			At siliqua formation stage		
		2019-20	2020-21	Mean	2019-20	2020-21	Mean
T ₁	Control(RF)	2.387 ± 0.003	2.413 ± 0.003	2.400 ^m	2.400 ± 0.006	2.430 ± 0.006	2.415 ⁿ
T ₂	HG@3.75kg/ha	2.187 ± 0.007	2.170 ± 0.006	2.178 ⁱ	2.210 ± 0.006	2.233 ± 0.003	2.221 ^j
T ₃	HG@5kg/ha	2.163 ± 0.003	2.183 ± 0.003	2.173 ^{hi}	2.193 ± 0.003	2.207 ± 0.003	2.200 ⁱ
T ₄	GK@15kg/ha	2.270 ± 0.006	2.293 ± 0.003	2.281 ^j	2.307 ± 0.009	2.327 ± 0.003	2.317 ^l
T ₅	GK@20kg/ha	2.247 ± 0.003	2.273 ± 0.003	2.260 ^j	2.280 ± 0.006	2.300 ± 0.012	2.290 ^k
T ₆	SA@100ppm	2.360 ± 0.006	2.367 ± 0.003	2.363 ^l	2.403 ± 0.009	2.417 ± 0.003	2.410 ⁿ
T ₇	SA@200ppm	2.300 ± 0.006	2.323 ± 0.003	2.311 ^k	2.343 ± 0.009	2.360 ± 0.006	2.351 ^m
T ₈	HG@3.75kg/ha+ SA@100ppm	2.010 ± 0.006	2.017 ± 0.003	2.013 ^e	2.053 ± 0.003	2.067 ± 0.003	2.060 ^e
T ₉	HG@3.75kg/ha+ SA@200ppm	2.037 ± 0.003	2.057 ± 0.009	2.047 ^f	2.077 ± 0.003	2.100 ± 0.006	2.088 ^f
T ₁₀	HG@5kg/ha+ SA@100ppm	1.897 ± 0.009	1.923 ± 0.003	1.910 ^c	1.953 ± 0.009	1.973 ± 0.003	1.963 ^c
T ₁₁	HG@5kg/ha+ SA@200ppm	1.833 ± 0.003	1.850 ± 0.012	1.841 ^b	1.877 ± 0.003	1.890 ± 0.006	1.883 ^b
T ₁₂	GK@15kg/ha+ SA@100ppm	2.140 ± 0.006	2.160 ± 0.006	2.150 ^h	2.193 ± 0.003	2.217 ± 0.007	2.205 ⁱ
T ₁₃	GK@15kg/ha+ SA@200ppm	2.070 ± 0.035	2.130 ± 0.006	2.100 ^g	2.143 ± 0.009	2.170 ± 0.006	2.156 ^h
T ₁₄	GK@20kg/ha+ SA@100ppm	2.077 ± 0.003	2.107 ± 0.003	2.092 ^g	2.100 ± 0.006	2.127 ± 0.009	2.113 ^g
T ₁₅	GK@20kg/ha+ SA@200ppm	1.953 ± 0.009	1.973 ± 0.003	1.963 ^d	2.003 ± 0.003	2.020 ± 0.006	2.011 ^d
T ₁₆	Irrigated(IR)	1.653 ± 0.003	1.677 ± 0.015	1.665 ^a	1.710 ± 0.006	1.750 ± 0.006	1.730 ^a
Mean		2.099	2.119	2.139	2.140	2.189	2.151
SE(m)		0.01	0.006	0.008	0.005	0.006	0.004
CD at 5% level of significance		0.028	0.019	0.023	0.016	0.017	0.011
CV (%)		0.793	0.529	0.516	0.435	0.461	0.236

**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

**Treatments with the same letters are not significantly different

all other treatments. The application of hydrogel at a rate of 5kg/ha, along with two foliar sprays of 200 ppm salicylic acid at the flowering and siliqua formation stages, resulted in a significant increase in Ψ_s . This was followed by the use of hydrogel@5kg/ha with two foliar sprays of 100 ppm salicylic acid at the flowering and siliqua formation stages. The highest Ψ_s were observed in the irrigated treatment at both sampling stages, in comparison to all other treatments. The levels of Ψ_s at the siliqua formation stage followed a similar trend as at the flowering stage, but the levels were more negative. The range of Ψ_s was ranging from 2.40 to 1.665 and 2.415 to 1.730 -MPa at the flowering and siliqua formation stages, respectively. At the siliqua formation stage, the control (rainfed) condition induced the maximum reduction in Ψ_s by 28.3%, while the application of HG@5Kg/ha+ SA@200ppm and HG@5Kg/ha+ SA@100ppm alleviated the reduction in Ψ_s by 22% and 18.7%, respectively as compared to rainfed conditions. The data prediction showed that GK treatments also improved the Ψ_s when compared with the control conditions.

4.2.7 Chlorophyll Fluorescence (Fv/Fm)

The data presented in Table 7 showed that the chlorophyll fluorescence of mustard plants during flowering was progressively reduced under rainfed conditions compared to all other treatments (Table 7). The application of hydrogel at a rate of 5kg/ha and two foliar sprays of salicylic acid at concentrations of 200ppm during the flowering and siliqua formation stages resulted in a notable increase in chlorophyll fluorescence, followed by HG@5kg/ha+ SA@100ppm. The highest levels of chlorophyll fluorescence were recorded in the irrigated treatment at both sampling stages, surpassing all other treatments. A similar trend for chlorophyll fluorescence was observed at the siliqua formation stage as at the flowering stage but at decreased values. Values were ranging from 0.635 to 0.810 and 0.596 to 0.773, respectively. At the siliqua formation stage, the greatest reduction in chlorophyll fluorescence was observed in the control (rainfed) treatment, with a decrease of 35.6%. However, this reduction was alleviated by 28.6 % and 23.9 %, respectively, through the application of HG@5kg/ha+SA@200ppm and HG@5kg/ha+ SA@100ppm as compared to the irrigated condition. The data presented in Table 7 showed that GK treatments also improved the chlorophyll fluorescence when compared with the control conditions.

4.2.8 Chlorophyll index

Observation of data revealed that at the flowering stage, the chlorophyll index was gradually reduced in rainfed conditions over all other corresponding treatments (Table 8). The application of hydrogel at a rate of 5 kg per hectare, combined with two foliar sprays of salicylic acid at concentrations of 100 ppm during both the flowering and siliqua formation stages, had a significant impact on the chlorophyll index, followed by HG@ 5kg/ha+ SA@200ppm. Among all the treatments, the highest chlorophyll index was observed in the irrigated group at both sampling stages.

Table 7: Effect of salicylic acid and hydrogel treatments on chlorophyll fluorescence (Fv/Fm) of Indian mustard variety RH 725 under rainfed conditions

Treatments		Chlorophyll Fluorescence (Fv/Fm)					
		At flowering stage			At siliqua formation stage		
		2019-20	2020-21	Mean	2019-20	2020-21	Mean
T ₁	Control(RF)	0.640 ± 0.006	0.630 ± 0.006	0.635 ^l	0.603 ± 0.003	0.590 ± 0.006	0.596 ^j
T ₂	HG@3.75kg/ha	0.707 ± 0.003	0.680 ± 0.006	0.693 ^{hi}	0.653 ± 0.003	0.640 ± 0.006	0.646 ^{gh}
T ₃	HG@5kg/ha	0.720 ± 0.006	0.703 ± 0.003	0.711 ^{gh}	0.660 ± 0.006	0.643 ± 0.003	0.651 ^g
T ₄	GK@15kg/ha	0.683 ± 0.003	0.657 ± 0.003	0.670 ^{ijk}	0.657 ± 0.009	0.617 ± 0.003	0.637 ^h
T ₅	GK@20kg/ha	0.690 ± 0.006	0.677 ± 0.003	0.683 ^{hij}	0.653 ± 0.003	0.627 ± 0.030	0.640 ^{gh}
T ₆	SA@100ppm	0.657 ± 0.003	0.643 ± 0.003	0.650 ^{kl}	0.610 ± 0.006	0.597 ± 0.003	0.603 ^j
T ₇	SA@200ppm	0.660 ± 0.006	0.653 ± 0.003	0.656 ^{kl}	0.633 ± 0.009	0.607 ± 0.003	0.620 ⁱ
T ₈	HG@3.75kg/ha+ SA@100ppm	0.767 ± 0.003	0.783 ± 0.003	0.775 ^{bcde}	0.717 ± 0.003	0.703 ± 0.003	0.710 ^{cd}
T ₉	HG@3.75kg/ha+ SA@200ppm	0.780 ± 0.006	0.797 ± 0.003	0.788 ^{abcd}	0.723 ± 0.003	0.707 ± 0.003	0.715 ^c
T ₁₀	HG@5kg/ha+ SA@100ppm	0.790 ± 0.006	0.817 ± 0.003	0.803 ^{ab}	0.747 ± 0.003	0.730 ± 0.006	0.738 ^b
T ₁₁	HG@5kg/ha+ SA@200ppm	0.793 ± 0.003	0.803 ± 0.007	0.798 ^{abc}	0.673 ± 0.019	0.723 ± 0.003	0.738 ^b
T ₁₂	GK@15kg/ha+ SA@100ppm	0.720 ± 0.006	0.753 ± 0.003	0.736 ^{fg}	0.683 ± 0.003	0.653 ± 0.003	0.668 ^f
T ₁₃	GK@15kg/ha+ SA@200ppm	0.737 ± 0.003	0.763 ± 0.003	0.750 ^{ef}	0.683 ± 0.003	0.660 ± 0.006	0.676 ^f
T ₁₄	GK@20kg/ha+ SA@100ppm	0.753 ± 0.003	0.773 ± 0.003	0.763 ^{def}	0.703 ± 0.003	0.680 ± 0.006	0.691 ^e
T ₁₅	GK@20kg/ha+ SA@200ppm	0.763 ± 0.003	0.773 ± 0.003	0.768 ^{cdef}	0.713 ± 0.003	0.683 ± 0.003	0.698 ^{de}
T ₁₆	Irrigated(IR)	0.800 ± 0.006	0.820 ± 0.006	0.810 ^a	0.790 ± 0.006	0.757 ± 0.003	0.773 ^a
Mean		0.728	0.734	0.731	0.686	0.663	0.675
SE(m)		0.004	0.004	0.011	0.007	0.004	0.004
CD at 5% level of significance		0.013	0.012	0.033	0.020	0.012	0.013
CV (%)		0.123	0.115	0.241	0.201	0.124	0.107

**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

**Treatments with the same letters are not significantly different

Table 8: Effect of salicylic acid and hydrogel treatments on chlorophyll index of Indian mustard variety RH 725 under rainfed conditions

Treatments		Chlorophyll index					
		At flowering stage			At siliqua formation stage		
		2019-20	2020-21	Mean	2019-20	2020-21	Mean
T ₁	Control(RF)	26.42 ± 0.19	25.41 ± 0.06	25.92 ^m	25.28 ± 0.14	24.53 ± 0.21	24.90 ^m
T ₂	HG@3.75kg/ha	31.54 ± 0.25	30.21 ± 0.06	30.87 ⁱ	28.58 ± 0.21	27.81 ± 0.05	28.19 ^j
T ₃	HG@5kg/ha	32.49 ± 0.17	31.64 ± 0.05	32.07 ^h	29.72 ± 0.07	28.65 ± 0.05	29.18 ⁱ
T ₄	GK@15kg/ha	29.52 ± 0.11	28.47 ± 0.15	29.00 ^{jk}	26.37 ± 0.12	25.67 ± 0.12	26.02 ^l
T ₅	GK@20kg/ha	30.36 ± 0.12	29.44 ± 0.06	29.90 ^{ij}	27.56 ± 0.07	26.58 ± 0.07	27.07 ^k
T ₆	SA@100ppm	28.28 ± 0.15	27.11 ± 0.06	27.69 ^l	25.13 ± 0.08	24.71 ± 0.02	24.92 ^m
T ₇	SA@200ppm	28.91 ± 0.03	27.88 ± 0.05	28.39 ^{kl}	26.38 ± 0.09	25.49 ± 0.16	25.94 ^l
T ₈	HG@3.75kg/ha+ SA@100ppm	37.26 ± 0.07	36.58 ± 0.07	36.92 ^{cd}	34.51 ± 0.11	34.52 ± 0.10	34.51 ^d
T ₉	HG@3.75kg/ha+ SA@200ppm	36.85 ± 0.07	35.23 ± 0.09	36.04 ^d	34.37 ± 0.12	33.37 ± 0.19	33.87 ^e
T ₁₀	HG@5kg/ha+ SA@100ppm	38.92 ± 0.03	37.88 ± 0.04	38.40 ^b	36.88 ± 0.06	36.25 ± 0.09	36.56 ^b
T ₁₁	HG@5kg/ha+ SA@200ppm	38.24 ± 0.10	37.28 ± 0.08	37.76 ^{bc}	35.23 ± 0.10	35.30 ± 0.10	35.26 ^c
T ₁₂	GK@15kg/ha+ SA@100ppm	33.21 ± 0.10	32.24 ± 0.09	32.72 ^{gh}	31.10 ± 0.05	30.33 ± 0.12	30.71 ^h
T ₁₃	GK@15kg/ha+ SA@200ppm	33.87 ± 0.05	32.77 ± 0.07	33.32 ^g	32.64 ± 0.105	31.61 ± 0.113	32.13 ^g
T ₁₄	GK@20kg/ha+ SA@100ppm	34.21 ± 0.11	33.56 ± 0.08	33.88 ^{ef}	33.55 ± 0.19	32.51 ± 0.09	33.03 ^f
T ₁₅	GK@20kg/ha+ SA@200ppm	35.40 ± 0.13	34.38 ± 0.13	34.89 ^e	34.45 ± 0.14	33.43 ± 0.13	33.94 ^e
T ₁₆	Irrigated(IR)	39.84 ± 0.10	41.51 ± 0.15	40.68 ^a	37.80 ± 0.08	36.59 ± 0.21	37.19 ^a
Mean		33.46	32.60	33.03	31.22	30.46	30.84
SE(m)		0.12	0.08	0.35	0.11	0.12	0.18
CD at 5% level of significance		0.36	0.25	1.07	0.31	0.36	0.55
CV (%)		0.54	0.47	1.52	0.61	0.70	0.84

**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

**Treatments with the same letters are not significantly different

Similar trends were observed at the siliqua formation stage, albeit at reduced levels. At the flowering and siliqua formation stages, the chlorophyll index ranged from 25.92 to 40.68 and 24.90 to 37.19, respectively.

The control (rainfed) condition resulted in a 49.3 % reduction in chlorophyll index at the siliqua formation stage, which was improved by 46.8 % and 41.5 % with the application of HG@5kg/ha+SA@100ppm and HG@5kg/ha+SA@200ppm, respectively. The results showed that GK treatments also improved the chlorophyll index when compared with the control conditions but this improvement is less than irrigated and hydrogel treatments.

4.2.9 Anthocyanin index

A perusal of data depicted in Table 9 revealed that at the flowering stage anthocyanin index of the mustard plant increased in rainfed conditions over all other corresponding treatments. The use of hydrogel at a rate of 5 kg per hectare, along with two foliar sprays of salicylic acid at 200 ppm and 100 ppm during the flowering and siliqua formation stages, resulted in a significant reduction in anthocyanin index. In comparison to other treatments, the anthocyanin index was highest in the rainfed treatment during both the flowering and siliqua formation stages. The results of the anthocyanin index at the siliqua formation stage followed a similar trend as at the flowering stage but with an increase in levels ranging from 0.026 to 0.165 and 0.040 to 0.188 at the flowering and siliqua formation stages, respectively. At the siliqua formation stage, the maximum increase in anthocyanin index was observed in the control (rainfed) condition with an increase of 78.7 %, which was then reduced by 69.6 % and 63.8 % due to the application of hydrogel HG@5kg/ha+SA@200ppm and HG@5kg/ha+SA@100ppm, respectively. The results showed that GK treatments also reduced the anthocyanin index when compared with the control conditions but have higher anthocyanin index when compared with the irrigated and hydrogel treatments.

4.2.10 Flavonol index

A critical evaluation of data depicted in Table 10 revealed that at the flowering stage, the flavonol index of the mustard plant increased in rainfed conditions over all other treatments. The application of hydrogel at a rate of 5kg/ha, along with two foliar sprays of salicylic acid at a concentration of 200 ppm and 100 ppm during the flowering and siliqua formation stages, resulted in a significant decrease in flavonol index. Rainfed treatment recorded the highest flavonol index at both the sampling stages among all the treatments. Similar trends for flavonol index were observed at the siliqua formation stage as compared to the flowering stage but with increasing levels. The range of flavonol index was between 0.530 to 0.783 and 0.548 to 0.827 at the flowering and siliqua formation stages, respectively. At the siliqua formation stage, the maximum increase in flavonol index was observed in the control condition (rainfed) by 33.7 %, which was further reduced by 31 % and 30.2 % due to the application of HG@5kg/ha+SA@200ppm and HG@5kg/ha+SA@200ppm, respectively.

Table 9: Effect of salicylic acid and hydrogel treatments on anthocyanin index of Indian mustard variety RH 725 under rainfed conditions

Treatments		Anthocyanin index					
		At flowering stage			At siliqua formation stage		
		2019-20	2020-21	Mean	2019-20	2020-21	Mean
T ₁	Control(RF)	0.170 ± 0.006	0.160 ± 0.012	0.165 ^a	0.197 ± 0.003	0.180 ± 0.012	0.188 ^a
T ₂	HG@3.75kg/ha	0.133 ± 0.003	0.123 ± 0.003	0.128 ^{de}	0.147 ± 0.003	0.137 ± 0.003	0.142 ^d
T ₃	HG@5kg/ha	0.117 ± 0.003	0.107 ± 0.003	0.112 ^f	0.137 ± 0.003	0.127 ± 0.003	0.132 ^e
T ₄	GK@15kg/ha	0.137 ± 0.003	0.130 ± 0.012	0.133 ^d	0.160 ± 0.006	0.153 ± 0.003	0.156 ^c
T ₅	GK@20kg/ha	0.127 ± 0.003	0.120 ± 0.006	0.123 ^e	0.147 ± 0.003	0.137 ± 0.003	0.142 ^d
T ₆	SA@100ppm	0.153 ± 0.003	0.150 ± 0.012	0.151 ^b	0.173 ± 0.007	0.170 ± 0.012	0.171 ^b
T ₇	SA@200ppm	0.143 ± 0.003	0.140 ± 0.012	0.141 ^c	0.167 ± 0.003	0.157 ± 0.003	0.162 ^c
T ₈	HG@3.75kg/ha+ SA@100ppm	0.077 ± 0.007	0.063 ± 0.003	0.070 ⁱ	0.083 ± 0.007	0.080 ± 0.006	0.081 ^j
T ₉	HG@3.75kg/ha+ SA@200ppm	0.063 ± 0.003	0.053 ± 0.009	0.058 ^j	0.077 ± 0.003	0.067 ± 0.003	0.072 ^k
T ₁₀	HG@5kg/ha+ SA@100ppm	0.047 ± 0.003	0.043 ± 0.003	0.045 ^k	0.073 ± 0.003	0.063 ± 0.003	0.068 ^k
T ₁₁	HG@5kg/ha+ SA@200ppm	0.043 ± 0.003	0.027 ± 0.003	0.035 ^l	0.057 ± 0.003	0.057 ± 0.007	0.057 ^l
T ₁₂	GK@15kg/ha+ SA@100ppm	0.113 ± 0.003	0.100 ± 0.012	0.106 ^f	0.123 ± 0.003	0.120 ± 0.012	0.121 ^f
T ₁₃	GK@15kg/ha+ SA@200ppm	0.103 ± 0.003	0.093 ± 0.003	0.098 ^g	0.120 ± 0.006	0.110 ± 0.012	0.115 ^g
T ₁₄	GK@20kg/ha+ SA@100ppm	0.090 ± 0.006	0.083 ± 0.003	0.086 ^h	0.107 ± 0.003	0.097 ± 0.003	0.102 ^h
T ₁₅	GK@20kg/ha+ SA@200ppm	0.077 ± 0.003	0.067 ± 0.003	0.072 ⁱ	0.097 ± 0.003	0.090 ± 0.006	0.093 ⁱ
T ₁₆	Irrigated(IR)	0.033 ± 0.003	0.020 ± 0.006	0.026 ^m	0.043 ± 0.003	0.037 ± 0.003	0.040 ^m
Mean		0.101	0.092	0.097	0.119	0.111	0.115
SE(m)		0.004	0.007	0.002	0.004	0.007	0.002
CD at 5% level of significance		0.012	0.022	0.006	0.012	0.019	0.006
CV (%)		0.690	0.139	0.279	0.599	0.102	0.254

**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

**Treatments with the same letters are not significantly different

Table 10: Effect of salicylic acid and hydrogel treatments on flavonol index of Indian mustard variety RH 725 under rainfed conditions

Treatments		Flavonol index					
		At flowering stage			At siliqua formation stage		
		2019-20	2020-21	Mean	2019-20	2020-21	Mean
T ₁	Control(RF)	0.763 ± 0.003	0.803 ± 0.009	0.783 ^a	0.817 ± 0.003	0.837 ± 0.003	0.827 ^a
T ₂	HG@3.75kg/ha	0.683 ± 0.003	0.693 ± 0.003	0.688 ^e	0.717 ± 0.003	0.737 ± 0.003	0.727 ^c
T ₃	HG@5kg/ha	0.653 ± 0.003	0.667 ± 0.003	0.660 ^f	0.693 ± 0.003	0.707 ± 0.003	0.700 ^d
T ₄	GK@15kg/ha	0.707 ± 0.003	0.733 ± 0.003	0.720 ^d	0.747 ± 0.006	0.770 ± 0.006	0.758 ^b
T ₅	GK@20kg/ha	0.687 ± 0.003	0.707 ± 0.009	0.697 ^e	0.723 ± 0.003	0.750 ± 0.006	0.736 ^c
T ₆	SA@100ppm	0.757 ± 0.003	0.777 ± 0.003	0.767 ^b	0.833 ± 0.033	0.813 ± 0.007	0.823 ^a
T ₇	SA@200ppm	0.727 ± 0.003	0.747 ± 0.003	0.737 ^c	0.763 ± 0.003	0.787 ± 0.003	0.775 ^b
T ₈	HG@3.75kg/ha+ SA@100ppm	0.573 ± 0.003	0.587 ± 0.007	0.580 ⁱ	0.600 ± 0.006	0.637 ± 0.003	0.618 ^g
T ₉	HG@3.75kg/ha+ SA@200ppm	0.547 ± 0.003	0.573 ± 0.003	0.560 ^j	0.587 ± 0.003	0.607 ± 0.003	0.597 ^h
T ₁₀	HG@5kg/ha+ SA@100ppm	0.537 ± 0.003	0.557 ± 0.003	0.547 ^{jk}	0.567 ± 0.003	0.587 ± 0.003	0.577 ⁱ
T ₁₁	HG@5kg/ha+ SA@200ppm	0.530 ± 0.006	0.543 ± 0.007	0.536 ^{kl}	0.557 ± 0.003	0.583 ± 0.003	0.570 ⁱ
T ₁₂	GK@15kg/ha+ SA@100ppm	0.653 ± 0.003	0.667 ± 0.003	0.660 ^f	0.693 ± 0.003	0.707 ± 0.003	0.700 ^d
T ₁₃	GK@15kg/ha+ SA@200ppm	0.633 ± 0.003	0.653 ± 0.003	0.643 ^g	0.667 ± 0.003	0.693 ± 0.003	0.680 ^e
T ₁₄	GK@20kg/ha+ SA@100ppm	0.610 ± 0.006	0.627 ± 0.003	0.618 ^h	0.647 ± 0.006	0.673 ± 0.003	0.660 ^f
T ₁₅	GK@20kg/ha+ SA@200ppm	0.583 ± 0.003	0.583 ± 0.003	0.583 ⁱ	0.617 ± 0.003	0.637 ± 0.006	0.627 ^g
T ₁₆	Irrigated(IR)	0.517 ± 0.003	0.543 ± 0.003	0.530 ^l	0.543 ± 0.007	0.553 ± 0.009	0.548 ^j
Mean		0.631	0.650	0.641	0.669	0.689	0.679
SE(m)		0.004	0.005	0.004	0.009	0.004	0.006
CD at 5% level of significance		0.011	0.014	0.013	0.027	0.013	0.018
CV (%)		0.105	0.131	0.987	0.242	0.112	0.126

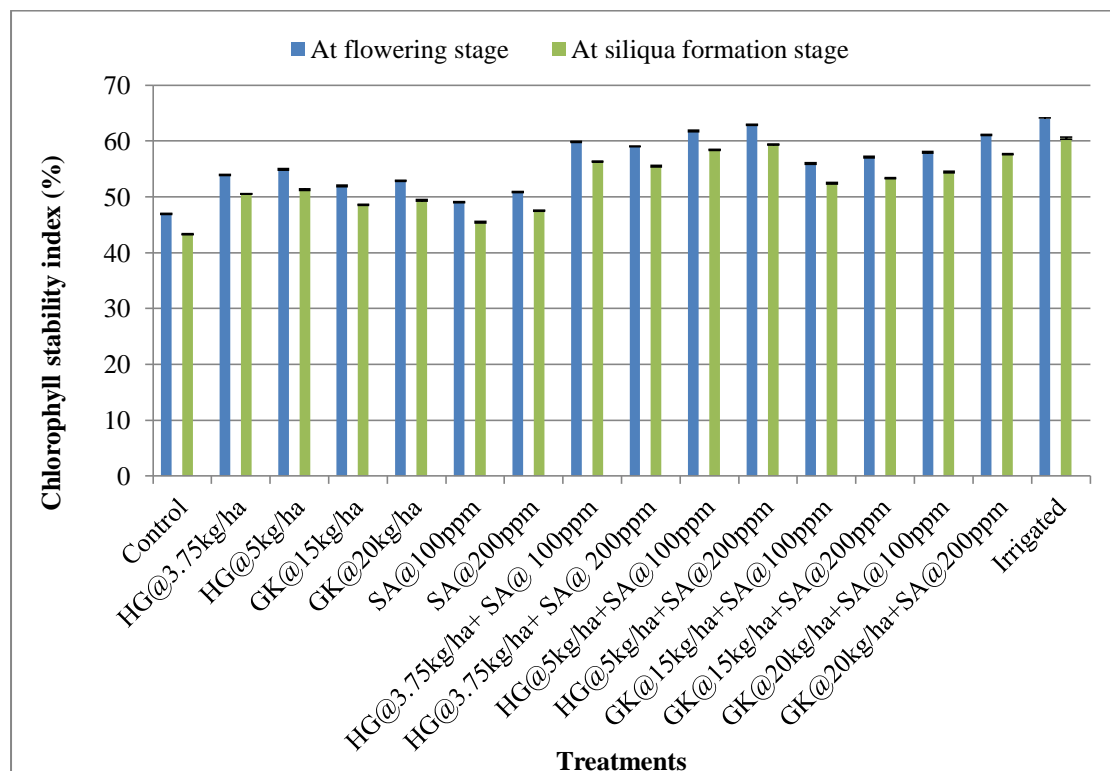
**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

***Treatments with the same letters are not significantly different

The results showed that GK treatments also reduced the flavonol index when compared with the control conditions.

4.2.11 Chlorophyll stability index (%)

Upon examination of data, it was discovered that during the flowering stage, the chlorophyll stability index was steadily diminished in the rainfed condition, compared to all other treatments (Figure 6). The application of hydrogel at a rate of 5kg/ha, along with two foliar sprays of 200 ppm salicylic acid at the flowering and siliqua formation stages, resulted in a significant increase in chlorophyll content, followed by the application of hydrogel at a rate of 3.75kg/ha, also with two foliar sprays of salicylic acid at the flowering and siliqua formation stages. In comparison with all other treatments, the irrigated treatment produced the highest chlorophyll stability index during both sampling stages. While the results at the siliqua formation stage followed a similar trend to that of the flowering stage but levels decreased. The percentage range was between 46.93% to 63.97% during flowering and 43.31% to 60.49% during siliqua formation. At the siliqua formation stage, the control (rainfed) condition experienced the greatest reduction in CSI, which amounted to 39.6%. However, applying hydrogel at 5kg/ha+SA (200ppm) and hydrogel at 3.75kg/ha+SA (200 ppm) improved CSI by 37% and 34.8%, respectively. The results showed that GK treatments also improved the CSI when compared with the control conditions but GK treatments showed less CSI when compared with irrigated and hydrogel treatments.



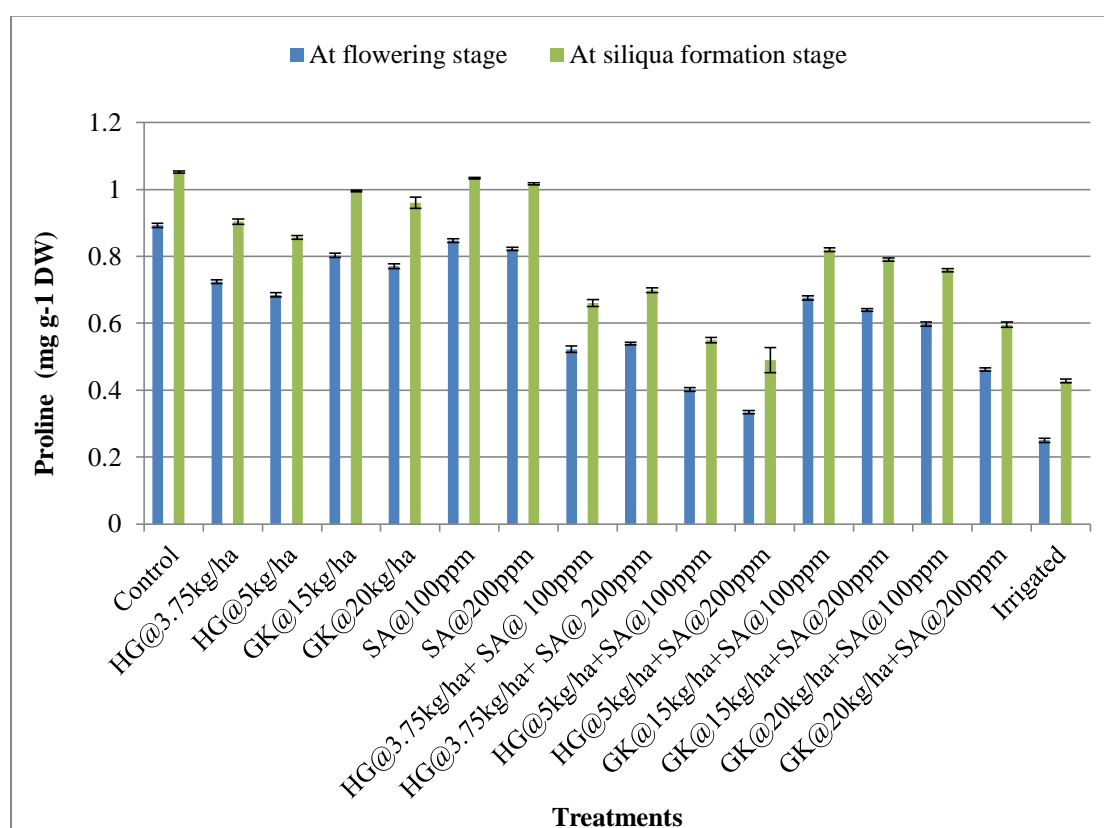
**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

Figure 6: Effect of salicylic acid and hydrogel treatments on chlorophyll stability index (%) of Indian mustard variety RH 725 under rainfed conditions (Values are means of two Rabi seasons (2019-20 and 2020-21) and vertical bars indicate standard error (\pm))

4.3 Biochemical parameters:

4.3.1 Proline content

Data showed that proline content in leaves at the flowering stage was significantly increased in rainfed conditions over all other treatments (Figure 7). The utilization of hydrogel@5kg/ha, accompanied by two foliar sprays of 200ppm and 100 ppm salicylic acid during the flowering and siliqua formation stages, resulting in a considerable decrease in the content of proline. The highest levels of proline content were recorded in the rainfed treatment, during both sampling stages, when compared to all other treatments. The levels of proline content during the siliqua formation stage followed a similar trend to those observed during the flowering stage but with increasing levels. The range was between 0.250 to 0.893 and 0.428 to 1.052 mg g⁻¹ DW at the flowering and siliqua formation stages, respectively.



**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

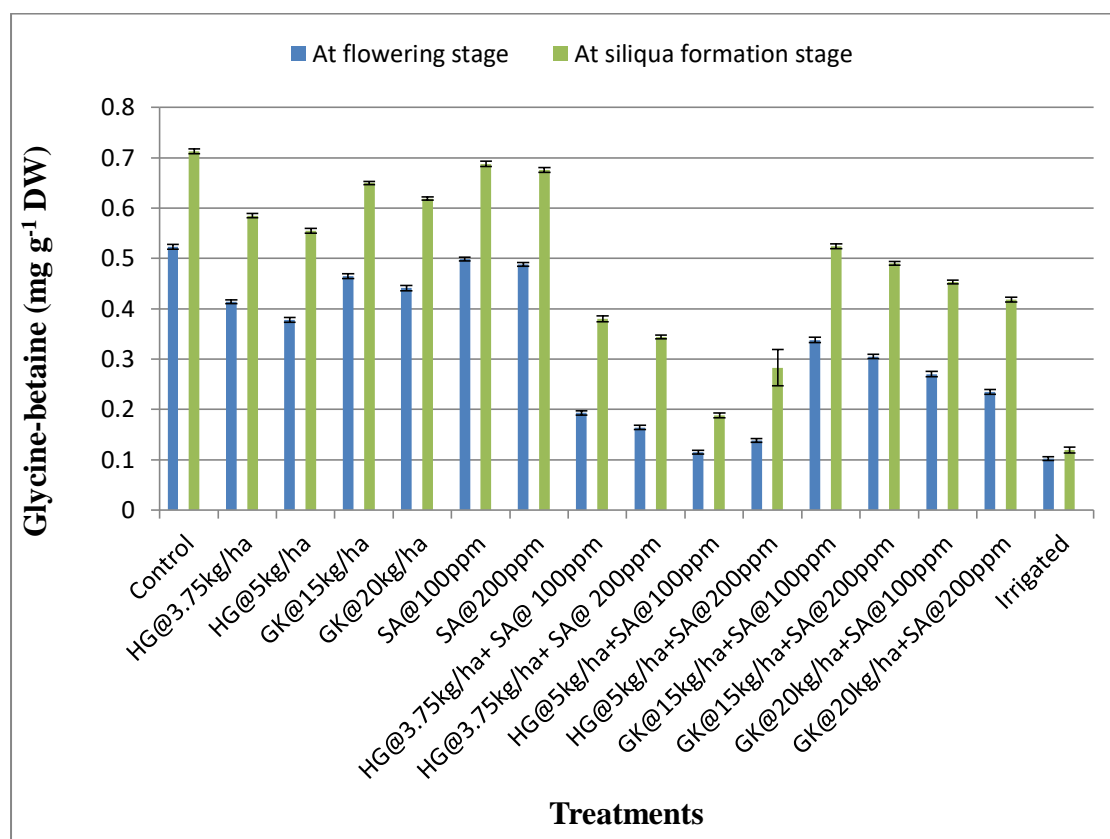
Figure 7: Effect of salicylic acid and hydrogel treatments on proline content (mg g⁻¹ DW) in the leaves of Indian mustard variety RH 725 under rainfed conditions (Values are means of two *Rabi* seasons (2019-20 and 2020-21) and vertical bars indicate standard error (±))

During the siliqua formation stage, the percentage of proline content increased to 57.9% under the control (rainfed) condition, whereas it was reduced by 51.8% and 45.9% with the application of hydrogel@5kg/ha+SA(200ppm) and hydrogel@5kg/ha+SA(100ppm), respectively, when compared to irrigated conditions. The results showed that GK treatments

also reduced the proline content when compared with the control conditions but showed more proline content when compared with irrigated and hydrogel treatments.

4.3.2 Glycine-Betaine content

A perusal of data revealed that glycine betaine content in leaves at the flowering stage was significantly increased in rainfed conditions over all other corresponding treatments (Figure 8). Glycine betaine content was notably reduced by the application of hydrogel@5kg/ha with two foliar sprays of 100ppm and 200 ppm salicylic acid during the flowering and siliqua formation stages. In comparison, the highest level of glycine betaine content was found in the rainfed treatment during both sampling stages.



**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

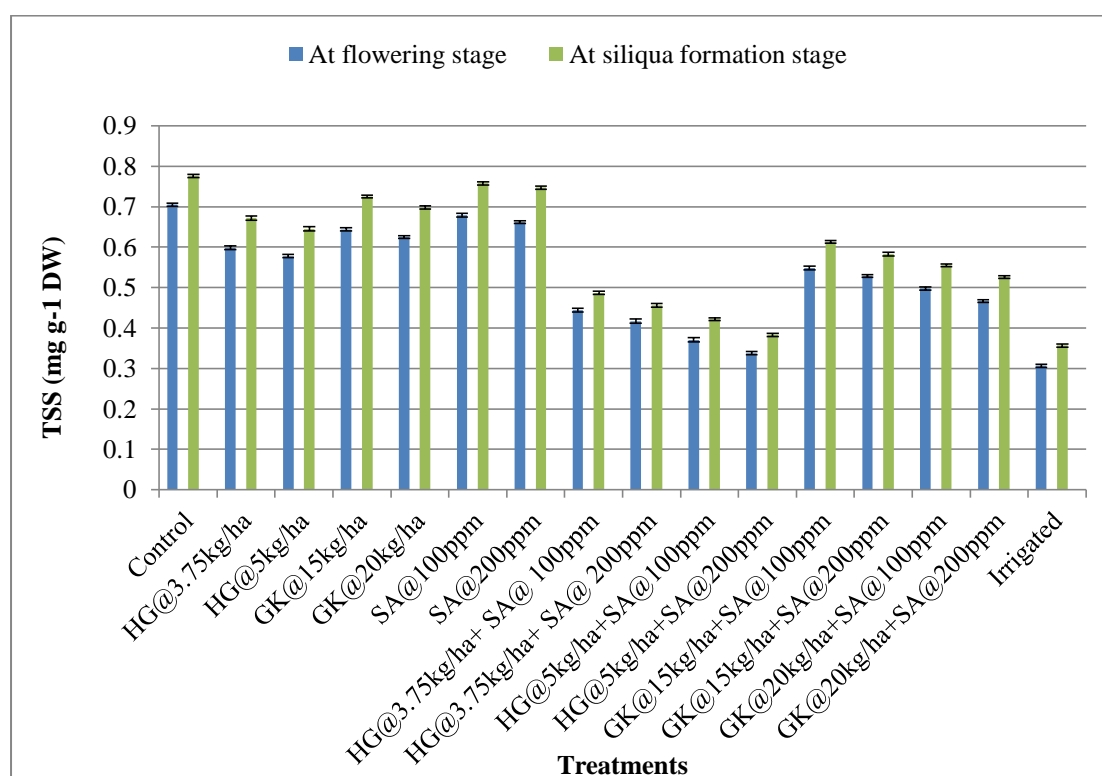
Figure 8: Effect of salicylic acid and hydrogel treatments on glycine-betaine content (mg g⁻¹ DW) in the leaves of Indian mustard variety RH 725 under rainfed conditions (Values are means of two *Rabi* seasons (2019-20 and 2020-21) and vertical bars indicate standard error (±))

The glycine betaine content at the siliqua formation stage followed a similar trend to that of the flowering stage, but with gradually increasing levels. It ranged from 0.102 to 0.523 and 0.119 to 0.713 mg g⁻¹ DW during the flowering and siliqua formation stages, respectively. At the siliqua formation stage, the percentage of glycine betaine content was increased by 82.3 % under rainfed control conditions, but it was reduced to 72.1 % and 58.1 % with the application of hydrogel@5kg/ha+SA (100 ppm) and hydrogel@5kg/ha+SA (200 ppm),

respectively when compared to irrigated conditions. The results showed that GK treatments also reduced the glycine betaine content when compared with the control conditions but showed more glycine betaine content when compared with irrigated and hydrogel treatments.

4.3.3 Total soluble sugar content (TSS)

Investigation of data clearly showed that TSS content in leaves at the flowering stage was significantly increased in rainfed conditions over all other treatments (Figure 9). By applying hydrogel@5kg/ha and two foliar sprays of 200ppm and 100 ppm salicylic acid at the flowering and siliqua formation stages, a considerable decrease in total soluble solids (TSS) content was observed.



**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

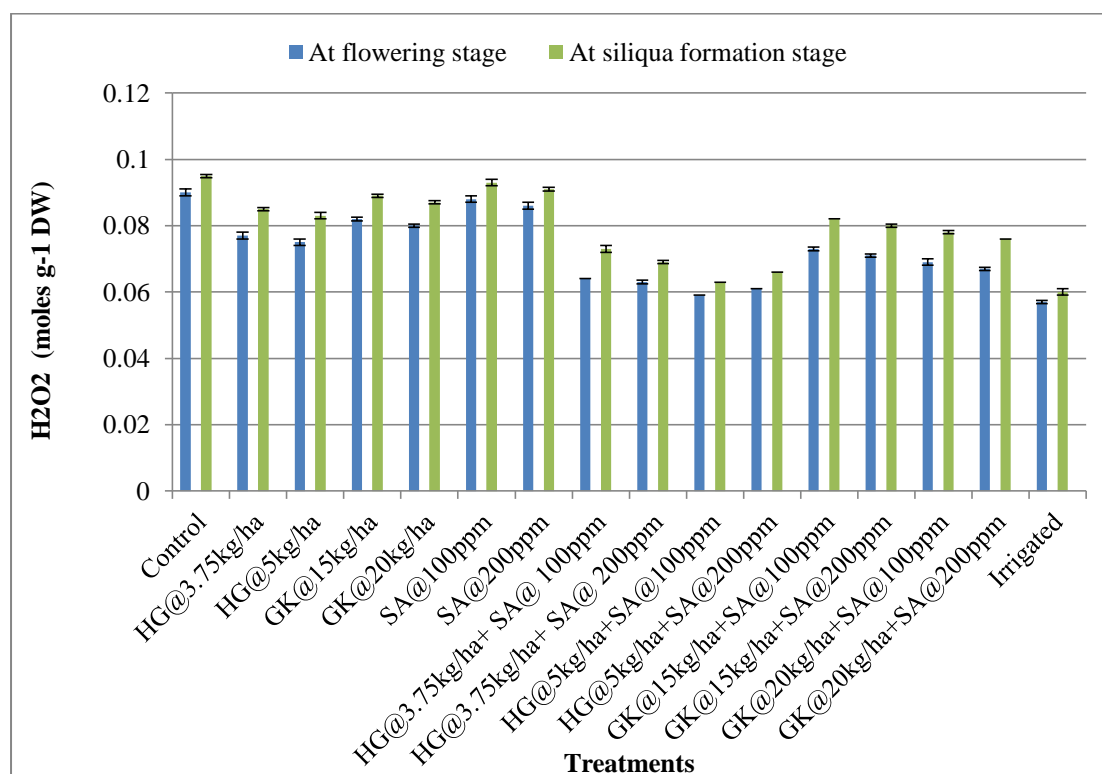
Figure 9: Effect of salicylic acid and hydrogel treatments on total soluble sugars (mg g⁻¹ DW) in the leaves of Indian mustard variety RH 725 under rainfed conditions (Values are means of two Rabi seasons (2019-20 and 2020-21) and vertical bars indicate standard error (±))

Out of all the treatments, the highest TSS content was recorded in the rainfed treatment at both sampling stages. Similar patterns were observed in TSS content at the siliqua formation stage as the flowering stage, with an increase in levels ranging from 0.307 to 0.705 and 0.357 to 0.776 mg g⁻¹ DW, respectively. At the siliqua formation stage, the TSS content was found to be 52.2 % higher in the control (rainfed) condition as compared to the irrigated treatment, which was reduced to 48.7 % and 43.5 % with the application of hydrogel@5kg/ha+SA (200ppm) and hydrogel@5kg/ha+SA (100ppm), respectively. The results showed that GK treatments also reduced the TSS content when compared with the

control conditions but showed more TSS content when compared with irrigated and hydrogel treatments.

4.3.4 Hydrogen peroxide (H₂O₂) content

Data depicted in Figure 10 showed that H₂O₂ content in leaves at the flowering stage was significantly increased in rainfed conditions over all other treatments. The application of hydrogel@5kg/ha, combined with two foliar sprays of 100 ppm and 200 ppm salicylic acid at both the flowering and silique formation stages, resulted in a significant reduction of H₂O₂ content. Among all treatments, the highest H₂O₂ content was observed in the rainfed treatment at both sampling stages.



**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

Figure 10: Effect of salicylic acid and hydrogel treatments on H₂O₂ content (moles g⁻¹ DW) in the leaves of Indian mustard variety RH 725 under rainfed conditions (Values are means of two *Rabi* seasons (2019-20 and 2020-21) and vertical bars indicate standard error (±))

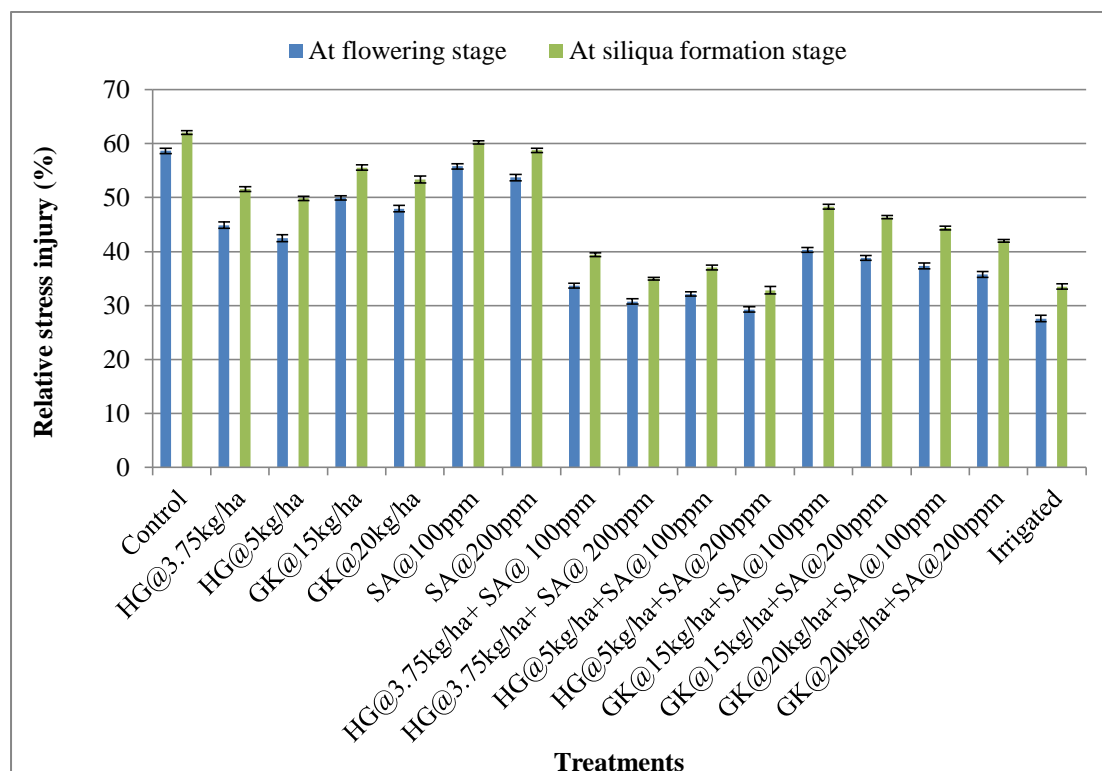
The H₂O₂ content was found to increase at the silique formation stage, ranging from 0.057 to 0.090 and 0.060 to 0.095 moles g⁻¹ DW at flowering and silique formation stages, respectively. At the silique formation stage, the control (rainfed) condition resulted in a 36.8 % induction of H₂O₂ content as compared to the irrigated treatment. However, the application of hydrogel@5kg/ha+SA (100 ppm) and hydrogel@5kg/ha+SA (200 ppm) reduced the H₂O₂ content to 33.6 % and 30.5 %, respectively. The results showed that GK treatments also reduced the H₂O₂ content when compared with the control conditions but showed more H₂O₂ content when compared with irrigated and hydrogel treatments.

4.4 Membrane injury indices

4.4.1 Relative stress injury (RSI %)

Persual of data revealed that RSI in leaves at the flowering stage was significantly increased in rainfed conditions over all other treatments (Figure 11).

The use of hydrogel at a rate of 5 kg per hectare, combined with two foliar sprays of 200 ppm salicylic acid during both the flowering and siliqua formation stages, resulted in a significant decrease in RSI, followed by hydrogel at a rate of 3.75 kg per hectare and two foliar sprays of 200 ppm salicylic acid during the flowering and siliqua formation stages.



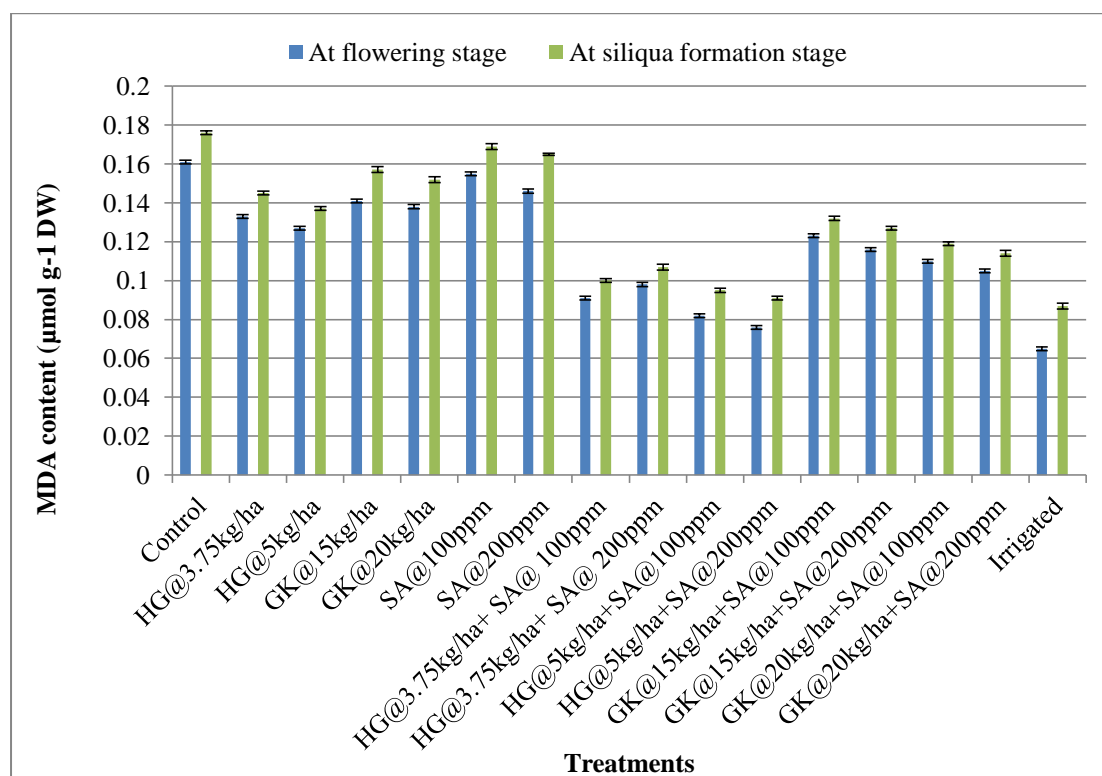
**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

Figure 11: Effect of salicylic acid and hydrogel treatments on relative stress injury (%) in the leaves of Indian mustard variety RH 725 under rainfed conditions (Values are means of two *Rabi* seasons (2019-20 and 2020-21) and vertical bars indicate standard error (\pm))

Maximum RSI was found in the rainfed treatment at both sampling stages, while similar RSI trends were observed during flowering and siliqua formation stages, but with increased levels. The percentages ranged from 27.60 % to 58.68 % and 33.51 % to 62.03 % during flowering and siliqua formation stages, respectively. At the siliqua formation stage, RSI increased to 45.9 % under rainfed conditions, while irrigation reduced to 47 % and 43.6 % with the application hydrogel@5kg/ha+SA (200 ppm) and hydrogel@3.75kg/ha+SA (200 ppm), respectively. when compared with the control conditions ,GK treatments also reduced RSI but this reduction was less than irrigated and hydrogel treatments.

4.4.2 Lipid peroxidation (MDA) content

A critical investigation of data revealed that MDA content in leaves at the flowering stage was significantly increased under rainfed conditions over all other corresponding treatments (Figure 12). The application of hydrogel@5kg/ha, along with two foliar sprays of 200 ppm and 100 ppm salicylic acid during the flowering and siliqua formation stages, resulted in a significant decrease in MDA content. In comparison to other treatments, the highest levels of MDA content were recorded in the rainfed treatment during both stages of sampling. A similar trend was followed at the siliqua formation stage as at the flowering stage, MDA content increased ranging from 0.065 to 0.161 and 0.087 to 0.176 $\mu\text{mol g}^{-1}$ DW at the flowering and siliqua formation stages, respectively. At the siliqua formation stage, MDA content increased by 47.2 % under control (rainfed) conditions compared to irrigated conditions, but this increase was reduced to 44.8 % and 42.4 % with the application of hydrogel@5kg/ha+SA (200 ppm) and hydrogel@5kg/ha+SA (100 ppm), respectively. The results showed that GK treatments also reduced the MDA content when compared with the control conditions but this reduction was less than irrigated and hydrogel treatments.



**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

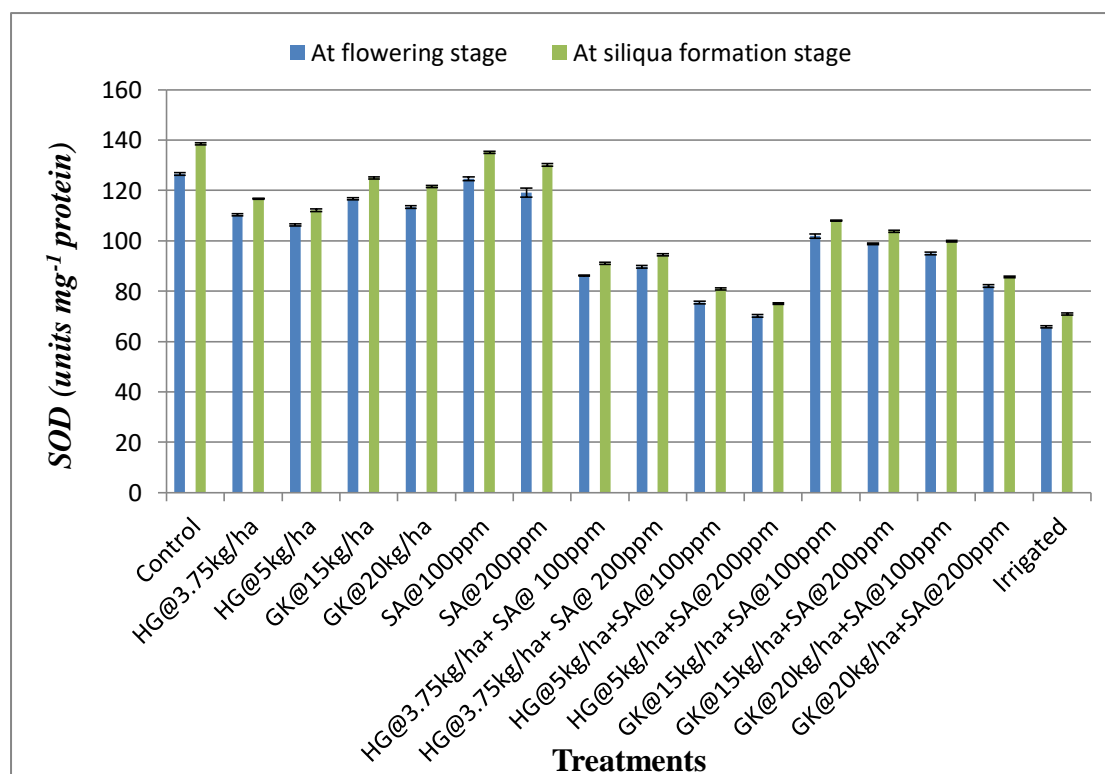
Figure 12: Effect of salicylic acid and hydrogel treatments on lipid peroxidation (MDA content) ($\mu\text{mol g}^{-1}$ DW) in the leaves of Indian mustard variety RH 725 under rainfed conditions (Values are means of two *Rabi* seasons (2019-20 and 2020-21) and vertical bars indicate standard error (\pm))

4.5 Antioxidant defence system

4.5.1 Enzymes (specific activity)

4.5.1.1 Superoxide dismutase (SOD)

A critical investigation of data revealed that at the flowering stage, rainfed conditions significantly enhanced the specific activity of SOD in leaves over irrigated treatment (Figure 13). The specific application of hydrogel@5kg/ha with two separate foliar sprays of 200 ppm and 100 ppm salicylic acid at the stages of flowering and siliqua formation demonstrated a gradual decrease in the specific activity of (SOD). Among all treatments, the maximum SOD activity was recorded during the rainfed treatment at both sampling stages. The results for SOD activity at the siliqua formation stage were consistent with those at the flowering stage but with increasing levels. Specifically, the range was from 65.84 to 126.47 and 70.86 to 138.52 units mg^{-1} protein at flowering and siliqua formation stages, respectively. At the siliqua formation stage, the specific activity of SOD increased by 48.8% under the control (rainfed) condition, while it decreased by 45.8% and 41.5% with the application of hydrogel@5kg/ha+SA(200ppm) and hydrogel@5kg/ha+SA(100ppm), respectively, when compared to the irrigated treatment. The results showed that GK treatments also decreased the specific activity of SOD when compared with the control conditions but this reduction was less than irrigated and hydrogel treatments.

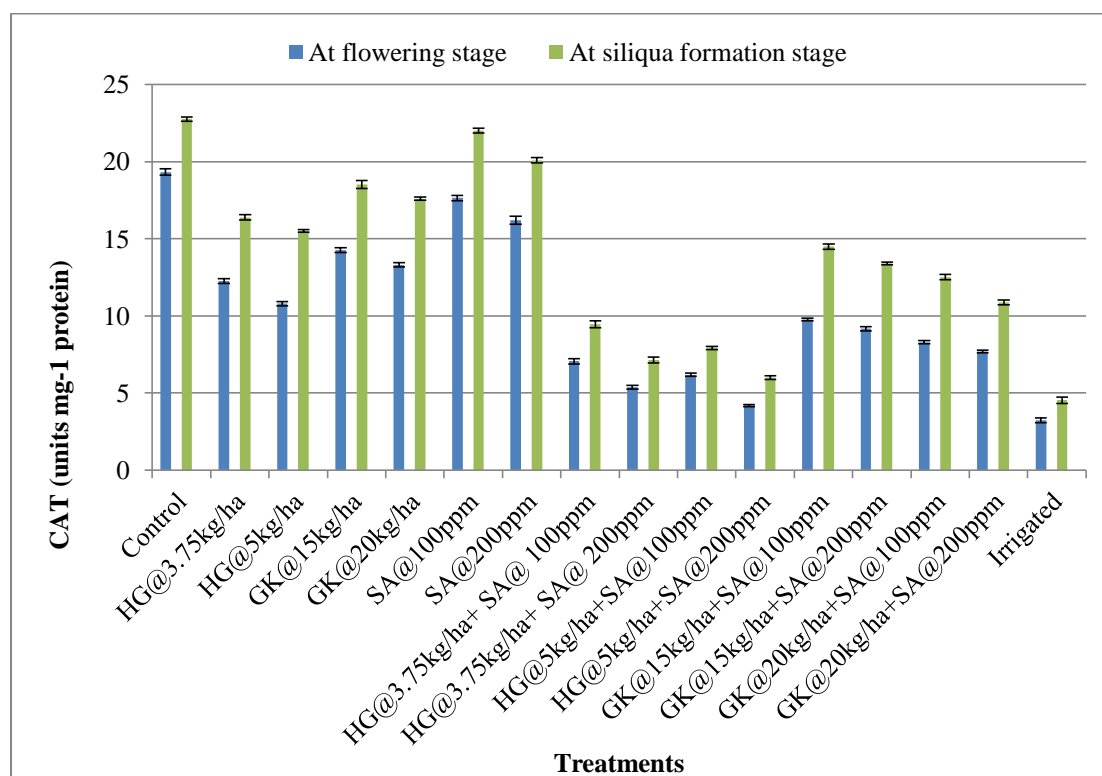


**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

Figure 13: Effect of salicylic acid and hydrogel treatments on the specific activity of superoxide dismutase (SOD) (units mg^{-1} protein) in the leaves of Indian mustard variety RH 725 under rainfed conditions (Values are means of two *Rabi* seasons (2019-20 and 2020-21) and vertical bars indicate standard error (\pm))

4.5.1.2 Catalase (CAT)

Investigation of data clearly showed that as similar to SOD, the specific activity of CAT in leaves was improved significantly at the flowering stage with rainfed conditions over irrigated treatment (Figure 14).



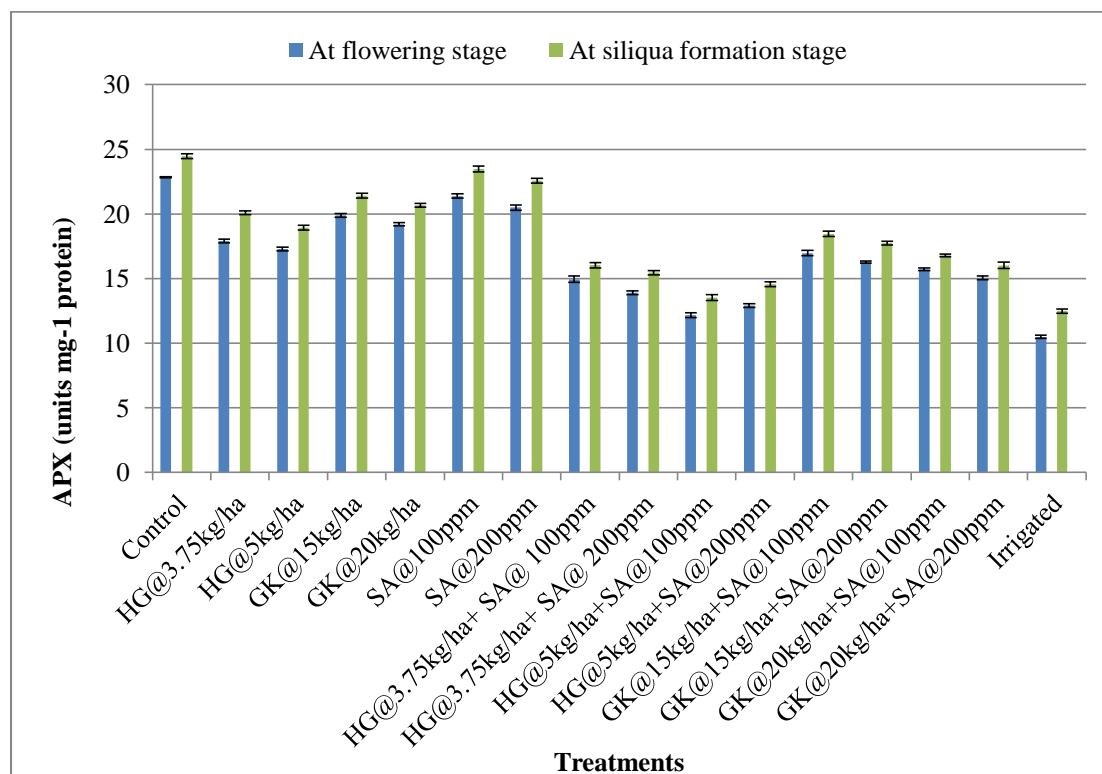
**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

Figure 14: Effect of salicylic acid and hydrogel treatments on the specific activity of catalase (CAT) (units mg⁻¹ protein) in the leaves of Indian mustard variety RH 725 under rainfed conditions (Values are means of two Rabi seasons (2019-20 and 2020-21) and vertical bars indicate standard error (±))

When hydrogel was applied at a rate of 5kg/ha, followed by two foliar sprays of 200ppm salicylic acid during the flowering and siliqua formation stages, the specific activity of CAT was observed to gradually decrease. In comparison to their respective treatments, maximum CAT activity was observed in the rainfed treatment at both sampling stages. The results for CAT activity at the siliqua formation stage followed a similar trend to those observed at the flowering stage, with increasing levels ranging from 3.23 to 19.32 and 4.53 to 22.76 units mg⁻¹ protein at the flowering and siliqua formation stages, respectively. At the siliqua formation stage, the specific activity of CAT showed an 80% increase under control (rainfed) conditions as compared to irrigated conditions, which were reduced by 73.6% and 68.7% with the application of hydrogel at a rate of 5kg/ha+SA(200ppm) and hydrogel at a rate of 3.75kg/ha+SA(200ppm), respectively. The results showed that GK treatments also decreased the specific activity of CAT when compared with the control conditions but this reduction was less than irrigated and hydrogel treatments.

4.5.1.3 Ascorbate peroxidase (APX)

Investigation of data clearly showed that as similar to APX, the specific activity of CAT in leaves was improved significantly at the flowering stage with rainfed conditions over irrigated treatment (Figure 15). The application of hydrogel at a rate of 5kg/ha, in conjunction with two foliar sprays of salicylic acid at 100ppm and 200 ppm during the flowering and siliqua formation stages, resulted in a gradual decline in the specific activity of APX.



**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

Figure 15: Effect of salicylic acid and hydrogel treatments on the specific activity of ascorbate peroxidase (APX) (units mg⁻¹ protein) in the leaves of Indian mustard variety RH 725 under rainfed conditions (Values are means of two Rabi seasons (2019-20 and 2020-21) and vertical bars indicate standard error (±))

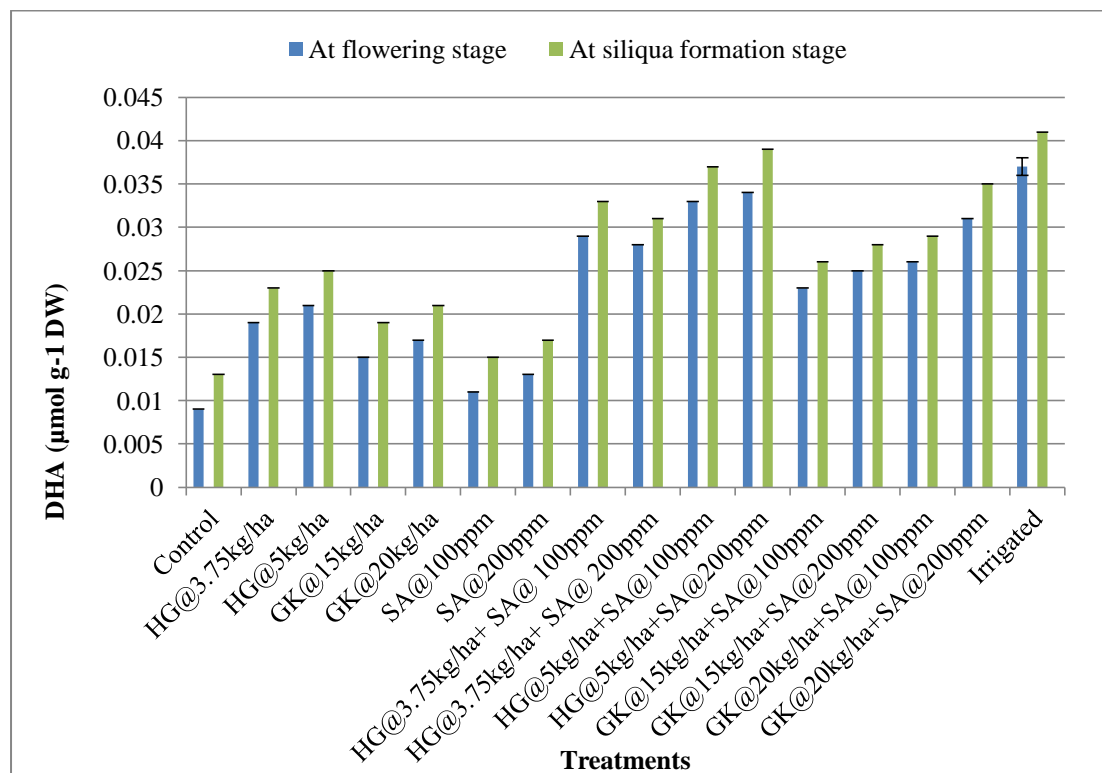
Among all the treatments, the rainfed treatment had the highest APX activity at both sampling stages. At the siliqua formation stage, similar trend for APX activity were observed as at the flowering stage, but with increasing levels ranging from 10.48 to 22.83 and 12.47 to 24.46 units mg⁻¹ protein at flowering and siliqua formation stages, respectively. At the siliqua formation stage, the specific activity of APX increased by 49 % under rainfed conditions when compared to irrigated conditions.

However, hydrogel@5kg/ha+SA (100 ppm) and hydrogel@ 5kg/ha+ SA(200ppm), reduced the specific activity of APX by 44.6% and 40.4%, respectively. The results showed that GK treatments also reduced the specific activity of APX when compared with the control conditions but this reduction was less than irrigated and hydrogel treatments.

4.5.2 Metabolites

4.5.2.1 Oxidized ascorbate (DHA)

Data depicted in Figure 16 clearly showed that DHA content in leaves decreased significantly at the flowering stage with rainfed conditions over irrigated treatment.



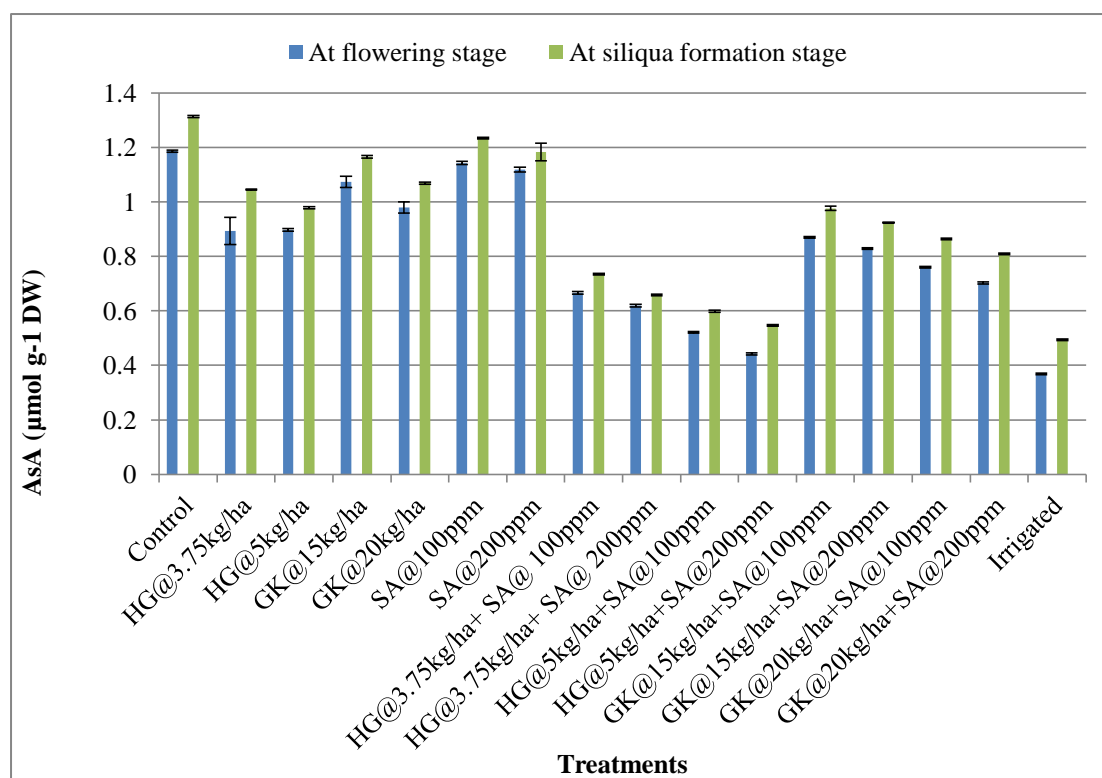
**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

Figure 16: Effect of salicylic acid and hydrogel treatments on oxidized ascorbate (DHA) ($\mu\text{mol g}^{-1}$ DW) in the leaves of Indian mustard variety RH 725 under rainfed conditions (Values are means of two *Rabi* seasons (2019-20 and 2020-21) and vertical bars indicate standard error (\pm))

The application of hydrogel at a rate of 5kg/ha, coupled with two foliar sprays of salicylic acid at 200ppm and 100 ppm during the flowering and siliqua formation stages, resulted in a significant increase in DHA content. In contrast to rainfed conditions, the maximum DHA content was found in the irrigated treatment at both the flowering and siliqua formation stages. The levels of DHA content ranged from 0.009 to 0.037 and 0.013 to 0.041 $\mu\text{mol g}^{-1}$ DW at the flowering and siliqua formation stages, respectively, with improved levels at the latter stage. At the siliqua formation stage, the DHA content was reduced to 4.2 fold under control conditions as compared to irrigated conditions. However, the application of hydrogel@5kg/ha+SA (200 ppm) and hydrogel@5kg/ha+SA (100 ppm) led to an increase in the DHA content to 3.7 and 3.4 fold, respectively. The results showed that GK treatments also increased the DHA content when compared with the control conditions but have less DHA content as compared to irrigated and hydrogel treatments.

4.5.2.2 Reduced ascorbate (AsA)

In contrast to DHA, AsA content in leaves was increased significantly at the flowering stage under rainfed conditions over irrigated treatment (Figure 17). The application of hydrogel at a rate of 5kg per hectare, along with two foliar sprays of salicylic acid at 200 ppm and 100 ppm during the flowering and siliqua formation stages resulted in a significant reduction in AsA content. The highest levels of AsA content were observed in the rainfed treatment at both flowering and siliqua formation stages. The trend at the siliqua formation stage was similar to that of the flowering stage, with increasing levels ranging from 0.369 to 1.187 and 0.494 to 1.313 $\mu\text{mol g}^{-1}$ DW at flowering and siliqua formation stages, respectively. At the siliqua formation stage, AsA content increased by 62.3 % under control (rainfed) conditions as compared to irrigated conditions, while it decreased by 58.3 % and 54.4 % with the application of hydrogel@5kg/ha+SA (200 ppm) and hydrogel@5kg/ha+SA (100 ppm), respectively. The results showed that GK treatments also reduced the AsA content when compared with the control conditions but this reduction is less than irrigated and hydrogel treatments.

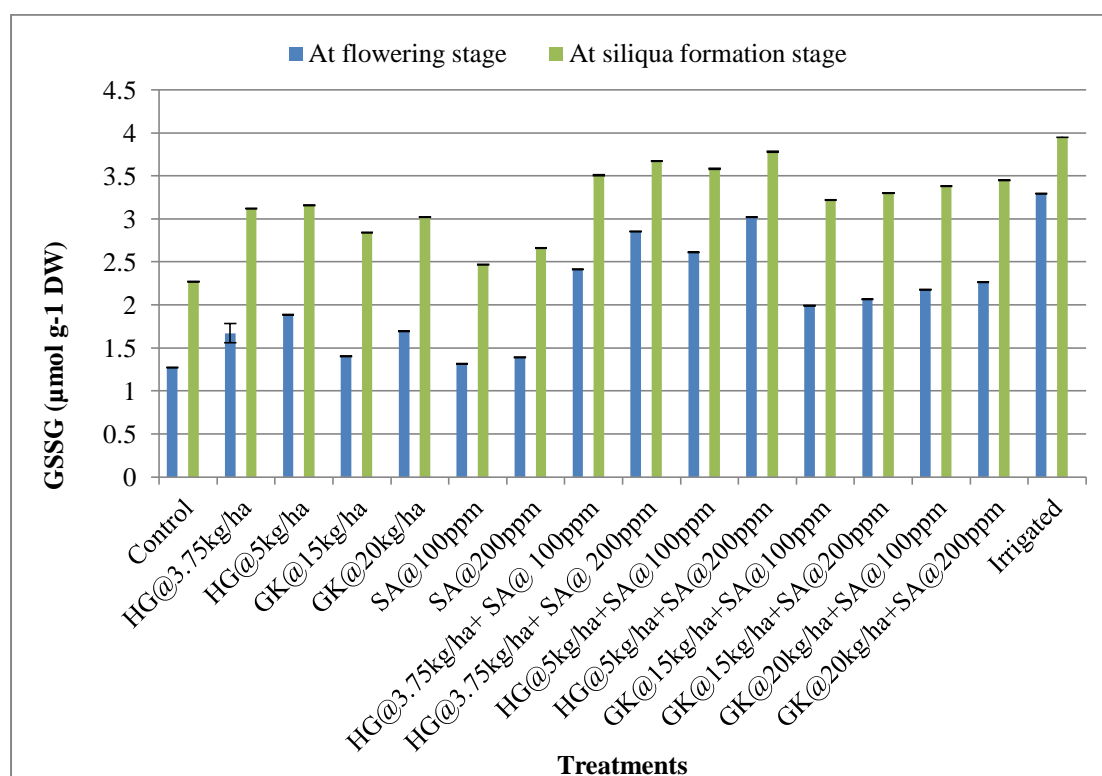


**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

Figure 17: Effect of salicylic acid and hydrogel treatments on reduced ascorbate (AsA) ($\mu\text{mol g}^{-1}$ DW) in the leaves of Indian mustard variety RH 725 under rainfed conditions (Values are means of two *Rabi* seasons (2019-20 and 2020-21) and vertical bars indicate standard error (\pm))

4.5.2.3 Oxidized glutathione (GSSG)

Data depicted in Figure 18 clearly showed that similar to DHA, GSSG content in leaves was decreased significantly at the flowering stage under rainfed conditions over irrigated treatment. The application of hydrogel@5kg/ha in combination with two foliar sprays of 200 ppm salicylic acid during the flowering and siliqua formation stage had a notable effect on the GSSG content, surpassing the corresponding treatments with hydrogel@3.75kg/ha+200 ppm SA. Notably, the highest levels of GSSG content were found in the irrigated treatment at both sampling stages, with the siliqua formation stage showing even more improvement than the flowering stage. The observed range of GSSG content was between 1.27 to 3.29 and 2.26 to 3.95 $\mu\text{mol g}^{-1}$ DW at flowering and siliqua formation stages, respectively. Under control (rainfed) conditions, GSSG content was reduced to 74.1 % at the siliqua formation stage, while it increased to 66.5 % and 61.7 % with the application of hydrogel@5kg/ha+SA (200 ppm) and hydrogel@3.75kg/ha+SA (200 ppm), respectively. The results showed that GK treatments also improved the GSSG content when compared with the control conditions but have less GSSG content when compared with the irrigated and hydrogel treatments.

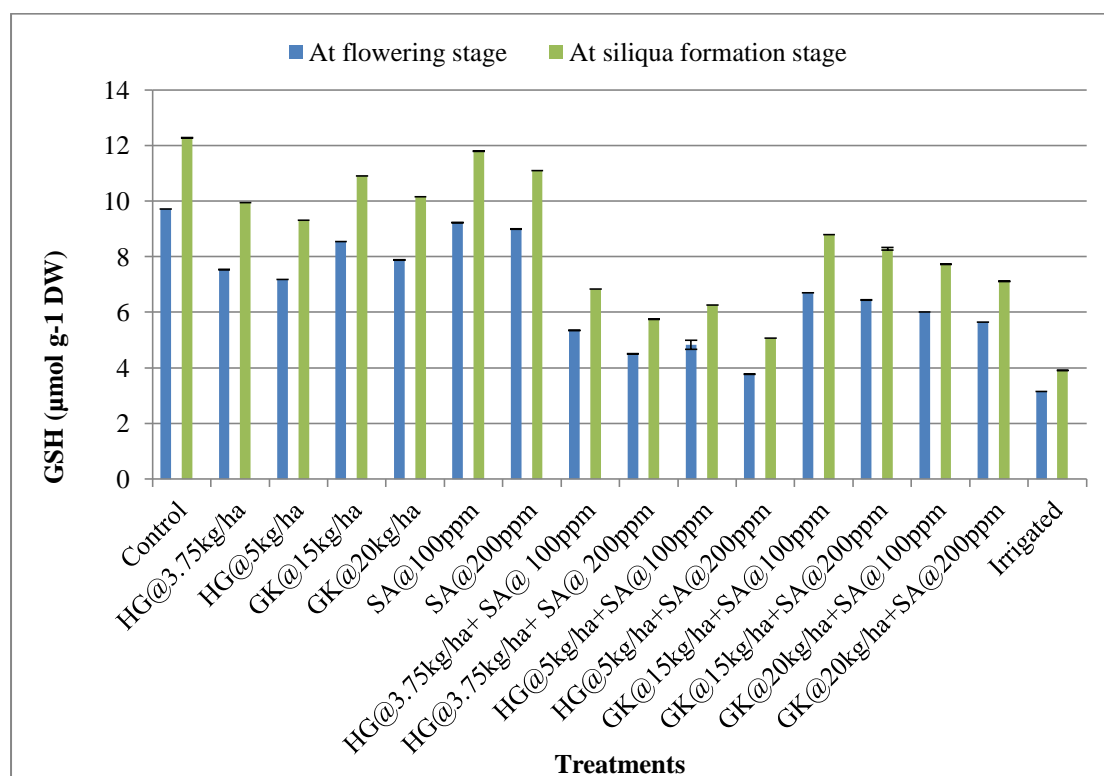


**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

Figure 18: Effect of salicylic acid and hydrogel treatments on oxidized glutathione (GSSG) ($\mu\text{mol g}^{-1}$ DW) in the leaves of Indian mustard variety RH 725 under rainfed conditions (Values are means of two *Rabi* seasons (2019-20 and 2020-21) and vertical bars indicate standard error (\pm))

4.5.2.4 Reduced glutathione (GSH)

Data showed that similar to AsA, GSH content in leaves was increased significantly at the flowering stage with rainfed conditions over irrigated treatment (Figure 19). The application of hydrogel at a rate of 5kg/ha, along with two foliar sprays of 200 ppm salicylic acid during the flowering and siliqua formation stages, resulted in a substantial decrease in GSH content, followed by hydrogel at a rate of 3.75kg/ha, with two foliar sprays of 200 ppm salicylic acid during the flowering and siliqua formation stages. The highest GSH content was recorded in the rainfed treatment at both the flowering and siliqua formation stages. The GSH content followed a similar trend at both stages but with increasing levels at the siliqua formation stage. The range at flowering and siliqua formation stages were 3.15 to 9.71 and 3.90 to 12.27 $\mu\text{mol g}^{-1}$ DW, respectively. At the siliqua formation stage, the GSH content increased to 68.1 % under rainfed conditions compared to irrigated conditions, which decreased to 58.7 % and 53.1 % with the application of hydrogel at a rate of 5kg/ha+SA(200 ppm) and hydrogel at a rate of 3.75kg/ha+SA(200 ppm), respectively. The results showed that GK treatments also decreased the GSH content when compared with the control conditions but this reduction is less than irrigated and hydrogel treatments.



**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

Figure 19: Effect of salicylic acid and hydrogel treatments on reduced glutathione (GSH) ($\mu\text{mol g}^{-1}$ DW) in the leaves of Indian mustard variety RH 725 under rainfed conditions (Values are means of two *Rabi* seasons (2019-20 and 2020-21) and vertical bars indicate standard error (\pm))

4.6 Yield and its attributes

4.6.1 Number of primary branches per plant

According to Table 11, the application of treatments had a significant impact on the number of primary branches per plant. The most effective treatment involved the use of hydrogel@5kg/ha along with two foliar sprays of 200ppm and 100 ppm salicylic acid during both the flowering and siliqua formation stages, resulting in a notable increase in the number of primary branches per plant. The control (rainfed) condition resulted in the lowest number of primary branches per plant (4.37), whereas irrigated conditions yielded the highest number of primary branches per plant (6.26). The application of hydrogel@5kg/ha+SA(200ppm) and hydrogel@5kg/ha+SA (100ppm) resulted in an improvement of primary branches per plant by 43% and 39.7%, respectively, whereas the control (rainfed) condition produced the highest reduction rate at 54.5%, as compared to the irrigated treatment. According to Table 11, GK treatments also increased the the number of primary branches per plant when compared with the control conditions.

4.6.2 Number of secondary branches per plant

The data presented in Table 11 indicates a significant decrease in the number of secondary branches per plant under rainfed conditions in comparison to irrigated treatment. However, the application of hydrogel@5kg/ha with two foliar sprays of 200 ppm and 100 ppm salicylic acid during the flowering and siliqua formation stages resulted in a marked improvement in the number of secondary branches per plant. Among all the treatments, the control (rainfed) treatment had the lowest number of secondary branches per plant (11.16) while the irrigated treatment had the highest number of secondary branches per plant (16.27). The maximum percent reduction in the number of secondary branches per plant was recorded at 45.7% in the control (rainfed) treatment, which was induced to 23.7% and 22.5% with the application of hydrogel@5kg/ha+SA (200ppm) and hydrogel@5kg/ha+SA (100ppm), respectively. The data presented in Table 11 showed that GK treatments also increased the number of secondary branches per plant when compared with the control conditions.

4.6.3 Main shoot length (cm)

The information presented in Table 12 clearly indicates a significant decrease in the length of the main shoot when subjected to rainfed conditions compared to irrigated treatment.

However, the application of hydrogel@5kg/ha with two foliar sprays of 200 ppm and 100 ppm salicylic acid during flowering and siliqua formation stage resulted in a gradual increase in main shoot length. Among the treatments, the lowest main shoot length of 61.89 cm was observed under rainfed conditions, whereas the highest main shoot length of 81.47 cm was observed under irrigated conditions. The maximum percentage reduction in main shoot length was 29.2 % as a result of the control (rainfed) condition, which was improved to 25.3 % and 23.1 % with the application of hydrogel@5kg/ha+SA (200 ppm) and hydrogel@5kg/ha+SA (100 ppm), respectively. The results showed that GK treatments also improved the main shoot length when compared with the control conditions but have shorter main shoot length when compared with irrigated and hydrogel treatments.

Table 11: Effect of salicylic acid and hydrogel treatments on the number of primary and secondary branches/plant of Indian mustard variety RH 725 under rainfed conditions

Treatments		Number of primary branches / plant			Number of secondary branches / plant		
		2019-20	2020-21	Mean	2019-20	2020-21	Mean
T ₁	Control(RF)	4.08 ± 0.03	4.67 ± 0.01	4.37 ^j	11.11 ± 0.02	11.21 ± 0.02	11.16 ⁱ
T ₂	HG@3.75kg/ha	4.82 ± 0.01	5.04 ± 0.09	4.93 ^{fg}	12.16 ± 0.01	13.06 ± 0.01	12.61 ^{fg}
T ₃	HG@5kg/ha	4.91 ± 0.01	5.12 ± 0.01	5.01 ^{efg}	12.25 ± 0.02	13.15 ± 0.01	12.70 ^{fg}
T ₄	GK@15kg/ha	4.64 ± 0.08	4.92 ± 0.02	4.78 ^{ghi}	11.95 ± 0.02	12.87 ± 0.07	12.41 ^g
T ₅	GK@20kg/ha	4.72 ± 0.03	4.98 ± 0.01	4.85 ^{gh}	12.06 ± 0.08	13.01 ± 0.08	12.54 ^{fg}
T ₆	SA@100ppm	4.25 ± 0.09	4.77 ± 0.02	4.51 ^{ij}	11.69 ± 0.03	11.43 ± 0.02	11.56 ^{hi}
T ₇	SA@200ppm	4.37 ± 0.05	4.84 ± 0.09	4.60 ^{hij}	11.79 ± 0.07	11.74 ± 0.04	11.77 ^h
T ₈	HG@3.75kg/ha+ SA@100ppm	5.47 ± 0.02	5.45 ± 0.06	5.46 ^d	12.89 ± 0.02	13.76 ± 0.02	13.32 ^{bcde}
T ₉	HG@3.75kg/ha+ SA@200ppm	5.94 ± 0.02	5.96 ± 0.02	5.95 ^{bc}	13.09 ± 0.03	13.86 ± 0.08	13.47 ^{bcd}
T ₁₀	HG@5kg/ha+ SA@100ppm	6.11 ± 0.02	6.12 ± 0.03	6.12 ^{bc}	13.33 ± 0.06	14.04 ± 0.03	13.68 ^{bc}
T ₁₁	HG@5kg/ha+ SA@200ppm	6.24 ± 0.03	6.27 ± 0.04	6.26 ^b	13.47 ± 0.01	14.15 ± 0.04	13.81 ^b
T ₁₂	GK@15kg/ha+ SA@100ppm	5.07 ± 0.09	5.19 ± 0.09	5.10 ^{efg}	12.33 ± 0.01	13.27 ± 0.03	12.79 ^{efg}
T ₁₃	GK@15kg/ha+ SA@200ppm	5.12 ± 0.02	5.26 ± 0.08	5.19 ^{def}	12.41 ± 0.04	13.38 ± 0.03	12.90 ^{defg}
T ₁₄	GK@20kg/ha+ SA@100ppm	5.31 ± 0.02	5.36 ± 0.05	5.33 ^{de}	12.52 ± 0.08	13.50 ± 0.03	13.01 ^{def}
T ₁₅	GK@20kg/ha+ SA@200ppm	5.78 ± 0.04	5.83 ± 0.038	5.81 ^c	12.63 ± 0.03	13.60 ± 0.02	13.12 ^{cdef}
T ₁₆	Irrigated(IR)	6.89 ± 0.05	6.64 ± 0.09	6.76 ^a	15.96 ± 0.01	16.58 ± 0.06	16.27 ^a
Mean		5.23	5.40	5.31	12.60	13.29	12.94
SE(m)		0.04	0.05	0.10	0.03	0.01	0.19
CD at 5% level of significance		0.04	0.04	0.32	0.01	0.03	0.59
CV (%)		0.46	0.48	2.88	0.04	0.14	2.16

**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

**Treatments with the same letters are not significantly different

Table 12: Effect of salicylic acid and hydrogel treatments on main shoot length (cm) and number of siliqua/plant of Indian mustard variety RH 725 under rainfed conditions

Treatments		Main shoot length (cm)			Number of siliqua/ Plant		
		2019-20	2020-21	Mean	2019-20	2020-21	Mean
T ₁	Control(RF)	61.50 ± 0.03	62.29 ± 0.08	61.89 ^m	198.66 ± 17.63	156.01 ± 4.16	177.33 ^o
T ₂	HG@3.75kg/ha	65.47 ± 0.15	67.29 ± 0.06	66.38 ^{hi}	245.66 ± 16.41	210.66 ± 14.52	228.16 ^j
T ₃	HG@5kg/ha	66.53 ± 0.10	68.27 ± 0.07	67.40 ^h	264.33 ± 9.28	219.00 ± 10.40	241.67 ⁱ
T ₄	GK@15kg/ha	63.25 ± 0.08	66.34 ± 0.11	64.80 ^{jk}	226.12 ± 7.03	194.66 ± 4.40	210.39 ^l
T ₅	GK@20kg/ha	64.10 ± 0.05	67.41 ± 0.10	65.76 ^{ij}	233.66 ± 14.51	204.01 ± 15.27	218.84 ^k
T ₆	SA@100ppm	62.04 ± 0.02	64.36 ± 0.13	63.20 ^l	207.66 ± 10.13	170.33 ± 11.66	189.00 ⁿ
T ₇	SA@200ppm	62.56 ± 0.05	65.42 ± 0.06	63.99 ^{kl}	215.20 ± 9.86	183.01 ± 12.58	199.10 ^m
T ₈	HG@3.75kg/ha+ SA@100ppm	75.51 ± 0.09	77.35 ± 0.09	76.43 ^c	311.33 ± 7.26	272.33 ± 4.66	291.83 ^e
T ₉	HG@3.75kg/ha+ SA@200ppm	73.75 ± 0.08	76.34 ± 0.07	75.04 ^d	322.66 ± 3.70	281.66 ± 7.26	302.16 ^d
T ₁₀	HG@5kg/ha+ SA@100ppm	76.50 ± 0.13	78.31 ± 0.13	77.40 ^c	335.34 ± 5.48	294.32 ± 4.41	314.83 ^c
T ₁₁	HG@5kg/ha+ SA@200ppm	78.40 ± 0.12	79.28 ± 0.05	78.84 ^b	346.67 ± 4.90	301.33 ± 4.42	324.00 ^b
T ₁₂	GK@15kg/ha+ SA@100ppm	68.54 ± 0.17	69.39 ± 0.19	68.97 ^g	273.33 ± 4.41	227.00 ± 5.85	250.17 ^h
T ₁₃	GK@15kg/ha+ SA@200ppm	70.67 ± 0.12	71.43 ± 0.13	71.05 ^f	280.00 ± 4.35	234.66 ± 5.48	257.33 ^h
T ₁₄	GK@20kg/ha+ SA@100ppm	71.45 ± 0.13	73.29 ± 0.13	72.37 ^e	294.33 ± 3.18	250.02 ± 8.66	272.17 ^g
T ₁₅	GK@20kg/ha+ SA@200ppm	72.47 ± 0.18	75.52 ± 0.03	74.02 ^d	303.66 ± 2.60	261.33 ± 7.26	282.50 ^f
T ₁₆	Irrigated(IR)	80.64 ± 0.07	82.30 ± 0.18	81.47 ^a	361.00 ± 23.18	317.33 ± 4.41	339.16 ^a
Mean		69.59	71.54	70.56	276.23	236.10	250.63
SE(m)		0.10	0.09	0.42	9.94	4.59	2.72
CD at 5% level of significance		0.28	0.27	1.29	7.87	6.32	8.28
CV (%)		0.21	0.20	0.75	1.28	1.01	1.50

**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

**Treatments with the same letters are not significantly different

4.6.4 Number of siliqua per plant

The examination of data revealed a significant decrease in the number of siliqua per plant under rainfed conditions as compared to irrigated treatment (as shown in Table 12). However, the application of hydrogel at a rate of 5kg/ha, accompanied by two foliar sprays of 200ppm and 100 ppm salicylic acid at the flowering and siliqua formation phases, resulting in a gradual increase in the number of siliqua. The maximum number of siliqua was observed in the irrigated treatment, which is greater than their respective treatments. Data ranging from 177.33 to 339.16 siliqua/plant. The highest percentage reduction in the number of siliqua per plant was recorded by 91.2% as a result of the control (rainfed) condition, which was induced by 82.7% and 77.5% with the application of hydrogel at 5kg/ha+SA(200ppm) and hydrogel at 5kg/ha+SA (100ppm), respectively. The results showed that GK treatments also increased the number of siliqua per plant when compared with the control conditions but have less number of siliqua per plant when compared with irrigated and hydrogel treatments.

4.6.5 Number of seeds per siliqua

Upon analyzing the data, it was revealed that the number of seeds per siliqua significantly decreased under rainfed conditions compared to irrigated treatment (Table 13). However, the application of hydrogel@5kg/ha followed by two foliar sprays of 100ppm and 200 ppm salicylic acid during the flowering and siliqua formation stages gradually increased the seeds per siliqua. Maximum seed count per siliqua was found in the irrigated treatment group, which surpassed all other corresponding treatments. Data ranging from 10.62 to 15.85 seeds/siliqua. Among all the treatments, the control (rainfed) treatment suffered the greatest reduction in seed count per siliqua, with a recorded decrease of 49.2 %. However, the application of hydrogel@5kg/ha+SA (100 ppm) and hydrogel@5kg/ha+SA (200 ppm) improved this decline by 34.6 % and 32.2 %, respectively. The results showed that GK treatments also increased the number of seeds per siliqua when compared with the control conditions but have less number of seeds per siliqua when compared with irrigated and hydrogel treatments.

4.6.6 1000 seed weight (g)

Upon critical analysis of the data, it was observed that the application of different treatment combinations significantly improved the weight of 1000 seeds as compared to rainfed conditions (Table 13). The weight of 1000 seeds gradually increased when hydrogel@5kg/ha was applied alongside two foliar sprays of 100 ppm and 200 ppm salicylic acid at flowering and siliqua formation stages. The highest weight of 1000 seeds was recorded in the irrigated treatment group. Data varied from 4.92 to 6.47g. Among all the treatments, SA@100 ppm treatment suffered the highest percentage reduction in 1000 seed weight, with a recorded decrease of 27.2 %. The application of hydrogel@5kg/ha+SA (100 ppm) and hydrogel@5kg/ha+SA (200 ppm) caused an increase of 17.9 % and 15.2 %, respectively. Additionally, the rainfed treatment group also showed improvement in the weight of 1000 seeds compared to using salicylic acid alone. The results showed that GK treatments also improved the 1000 seed weight when compared with the control conditions but this improvement is less than irrigated and hydrogel treatments.

Table 13: Effect of salicylic acid and hydrogel treatments on the number of seeds/ siliqua and 1000-seed weight (g) of Indian mustard variety RH 725 under rainfed conditions

Treatments		Number of seeds/ Siliqua			1000-seed weight(g)		
		2019-20	2020-21	Mean	2019-20	2020-21	Mean
T ₁	Control (RF)	10.06 ± 0.08	11.17 ± 0.04	10.62 ^l	4.97 ± 0.04	5.20 ± 0.08	5.09 ^{klm}
T ₂	HG@3.75kg/ha	10.98 ± 0.02	12.13 ± 0.07	11.56 ^{hi}	5.13 ± 0.09	5.49 ± 0.02	5.30 ^{hijk}
T ₃	HG@5kg/ha	11.12 ± 0.04	12.21 ± 0.02	11.67 ^h	5.15 ± 0.09	5.57 ± 0.02	5.36 ^{ghij}
T ₄	GK@15kg/ha	10.69 ± 0.02	11.91 ± 0.04	11.30 ^j	5.01 ± 0.09	5.28 ± 0.01	5.14 ^{ijklm}
T ₅	GK@20kg/ha	10.85 ± 0.03	12.02 ± 0.02	11.44 ^{ij}	5.06 ± 0.02	5.38 ± 0.02	5.22 ^{ijkl}
T ₆	SA@100ppm	10.24 ± 0.02	11.48 ± 0.09	10.86 ^k	4.82 ± 0.01	5.02 ± 0.07	4.92 ^m
T ₇	SA@200ppm	10.35 ± 0.05	11.73 ± 0.08	11.04 ^k	4.89 ± 0.02	5.13 ± 0.02	5.01 ^{lm}
T ₈	HG@3.75kg/ha+ SA@100ppm	11.76 ± 0.03	12.96 ± 0.03	12.36 ^e	5.47 ± 0.07	6.06 ± 0.08	5.76 ^{de}
T ₉	HG@3.75kg/ha+ SA@200ppm	12.09 ± 0.09	13.49 ± 0.07	12.79 ^d	5.59 ± 0.02	6.13 ± 0.04	5.86 ^{cd}
T ₁₀	HG@5kg/ha+ SA@100ppm	13.72 ± 0.04	14.87 ± 0.03	14.29 ^b	5.74 ± 0.03	6.26 ± 0.06	6.05 ^{bc}
T ₁₁	HG@5kg/ha+ SA@200ppm	13.42 ± 0.01	14.65 ± 0.02	14.04 ^c	5.86 ± 0.03	6.39 ± 0.09	6.12 ^b
T ₁₂	GK@15kg/ha+ SA@100ppm	11.31 ± 0.03	12.47 ± 0.09	11.89 ^g	5.17 ± 0.02	5.67 ± 0.08	5.42 ^{ghi}
T ₁₃	GK@15kg/ha+ SA@200ppm	11.44 ± 0.01	12.62 ± 0.03	12.03 ^{fg}	5.22 ± 0.05	5.77 ± 0.01	5.49 ^{gh}
T ₁₄	GK@20kg/ha+ SA@100ppm	11.55 ± 0.03	12.74 ± 0.09	12.15 ^f	5.28 ± 0.09	5.86 ± 0.08	5.57 ^{efg}
T ₁₅	GK@20kg/ha+ SA@200ppm	11.95 ± 0.06	13.25 ± 0.08	12.60 ^d	5.36 ± 0.05	5.96 ± 0.03	5.65 ^{def}
T ₁₆	Irrigated(IR)	15.46 ± 0.14	16.24 ± 0.07	15.85 ^a	6.14 ± 0.03	6.81 ± 0.06	6.47 ^a
Mean		11.69	12.87	12.28	5.30	5.75	5.52
SE(m)		0.04	0.02	0.06	0.08	0.01	0.07
CD at 5% level of significance		0.12	0.05	0.20	0.02	0.04	0.23
CV (%)		0.67	0.27	0.81	0.25	0.42	1.95

**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

**Treatments with the same letters are not significantly different

4.6.7 Seed yield (kg/ha)

Data demonstrate that seed yield is notably diminished under rainfed conditions compared to irrigated treatments (Table 14). However, when hydrogel@5kg/ha is applied in conjunction with two foliar sprays of 200ppm and 100 ppm salicylic acid during flowering and siliqua formation stages, seed yield is significantly increased. The lowest seed yield was recorded in the rainfed treatment and was consistently lower than the corresponding treatments. Data ranged from 1871 to 2365 kg/ha. The control (rainfed) treatment induced the highest percent reduction in seed yield, which was recorded at 26.4%. However, the application of hydrogel@5kg/ha+SA (200ppm) and hydrogel@5kg/ha+SA (100ppm) increased seed yield by 15.4% and 14.6%, respectively. The results showed that GK treatments also improved the seed yield when compared with the control conditions but GK treatments have less seed yield when compared with the irrigated and hydrogel treatments.

4.6.8 Biological yield (kg/ha)

The data presented in Table 14 shows a significant decrease in biological yield under rainfed conditions when compared to irrigated treatment. However, the application of hydrogel@5kg/ha with two foliar sprays of 200ppm and 100 ppm salicylic acid during flowering and siliqua formation stages led to a significant increase in biological yield. The lowest biological yield was observed in the rainfed treatment and ranged from 6777 to 7883 kg/ha across all corresponding treatments. The maximum percentage reduction in biological yield was recorded at 16.3% in the control (rainfed) condition, which was improved by 14.2% and 13.7% with the application of hydrogel@5kg/ha+SA (200ppm) and hydrogel@5kg/ha+SA (100ppm), respectively. The results showed that GK treatments also increased the biological yield when compared with the control conditions but GK treatments have less biological yield when compared with the irrigated and hydrogel treatments.

4.6.9 Stover yield (kg/ha)

Upon examining the data presented in Table 15, it becomes apparent that the yield of stover was noticeably diminished under rainfed conditions when compared to irrigated treatment. However, the application of hydrogel@5kg/ha, accompanied by two foliar sprays of salicylic acid at both the flowering and siliqua formation stages, resulted in a significant increase in stover yield. This was most evident with hydrogel@5kg/ha+SA (200 ppm), followed by hydrogel@5kg/ha+SA (100 ppm), in comparison to the control treatment. The lowest stover yield was observed in the rainfed treatment and ranged from 4906 to 5559 kg/ha across all corresponding treatments. The greatest percentage reduction in stover yield was observed in the control (rainfed) treatment, with a decrease of 12.4 %, as opposed to 13.3 % and 13.1 % increases observed with hydrogel@5kg/ha+SA (200 ppm) and hydrogel@5kg/ha+SA (100 ppm), respectively. The results showed that GK treatments also improved the stover yield when compared with the control conditions but GK treatments have less stover yield when compared with the irrigated and hydrogel treatments.

Table 14: Effect of salicylic acid and hydrogel treatment on seed yield (kg/ha) and biological yield (kg/ha) of Indian mustard variety RH 725 under rainfed conditions

Treatments		Seed yield((kg/ha)			Biological yield(kg/ha)		
		2019-20	2020-21	Mean	2019-20	2020-21	Mean
T ₁	Control(RF)	1851 ± 10.16	1890 ± 6.01	1871 ^P	6711 ± 21.69	6843. ± 17.38	6777 ^P
T ₂	HG@3.75kg/ha	1982 ± 4.04	2005 ± 19.14	1993 ^k	7050 ± 10.69	7135 ± 30.50	7092 ^k
T ₃	HG@5kg/ha	2008 ± 5.76	2023 ± 9.03	2016 ^j	7059 ± 13.00	7223 ± 15.30	7141 ^j
T ₄	GK@15kg/ha	1932 ± 6.08	1957 ± 8.42	1944 ^m	6839 ± 15.63	6925 ± 17.23	6882 ⁿ
T ₅	GK@20kg/ha	1961 ± 5.93	1979 ± 8.99	1970 ^l	6923 ± 16.21	7021 ± 17.43	6972 ^l
T ₆	SA@100ppm	1885 ± 11.67	1912 ± 6.63	1899 ^o	6766 ± 24.91	6871 ± 19.09	6819 ^o
T ₇	SA@200ppm	1907 ± 6.01	1934 ± 5.99	1921 ⁿ	6866 ± 14.22	6920 ± 14.22	6893 ^m
T ₈	HG@3.75kg/ha+ SA@100ppm	2100 ± 5.29	2143 ± 6.35	2121 ^e	7569 ± 12.75	7651 ± 17.64	7610 ^e
T ₉	HG@3.75kg/ha+ SA@200ppm	2124 ± 4.03	2165 ± 5.50	2144 ^d	7537 ± 12.36	7726 ± 12.76	7632 ^d
T ₁₀	HG@5kg/ha+ SA@100ppm	2140 ± 13.31	2179 ± 8.42	2159 ^c	7627 ± 20.28	7795 ± 16.86	7711 ^c
T ₁₁	HG@5kg/ha+ SA@200ppm	2172 ± 9.62	2198 ± 11.57	2185 ^b	7690 ± 17.13	7800 ± 19.92	7745 ^b
T ₁₂	GK@15kg/ha+ SA@100ppm	2030 ± 13.22	2047 ± 8.44	2038 ⁱ	7229 ± 26.72	7320. ± 16.52	7274 ⁱ
T ₁₃	GK@15kg/ha+ SA@200ppm	2051 ± 7.25	2079 ± 5.82	2065 ^h	7320 ± 16.16	7396. ± 15.63	7358 ^b
T ₁₄	GK@20kg/ha+ SA@100ppm	2069 ± 5.29	2094 ± 8.71	2081 ^g	7386 ± 14.57	7483 ± 15.86	7435 ^g
T ₁₅	GK@20kg/ha+ SA@200ppm	2083 ± 11.93	2120 ± 5.22	2101 ^f	7410 ± 20.32	7571 ± 11.67	7491 ^f
T ₁₆	Irrigated(IR)	2320 ± 8.38	2410 ± 9.53	2365 ^a	7755 ± 17.07	8011 ± 19.32	7883 ^a
Mean		2038	2071	2055	7233	7356	7295
SE(m)		5.07	4.12	4.90	4.89	5.10	5.21
CD at 5% level of significance		12.72	10.95	8.23	11.20	13.80	10.11
CV (%)		0.39	0.31	0.50	0.11	0.12	0.18

**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

**Treatments with the same letters are not significantly different

Table 15: Effect of salicylic acid and hydrogel treatments on stover yield (kg/ha) and Harvest index (%) of Indian mustard variety RH 725 under rainfed conditions

Treatments		Stover yield (kg/ha)			Harvest index (%)		
		2019-20	2020-21	Mean	2019-20	2020-21	Mean
T ₁	Control(RF)	4860 ± 8.32	4952 ± 11.37	4906 ^p	27.58 ± 0.11	27.62 ± 0.06	27.60 ^f
T ₂	HG@3.75kg/ha	5068 ± 6.65	5130 ± 13.07	5099 ^k	28.11 ± 0.01	28.10 ± 0.14	28.10 ^{bcd}
T ₃	HG@5kg/ha	5051 ± 7.16	5199 ± 6.44	5125 ^j	28.44 ± 0.02	28.01 ± 0.06	28.22 ^{bc}
T ₄	GK@15kg/ha	4907 ± 9.56	4968 ± 8.96	4937 ⁿ	28.24 ± 0.03	28.26 ± 0.04	28.25 ^b
T ₅	GK@20kg/ha	4962 ± 10.58	5042 ± 8.36	5002 ^l	28.33 ± 0.09	28.18 ± 0.05	28.26 ^b
T ₆	SA@100ppm	4881 ± 13.22	4959 ± 12.41	4920 ^o	27.86 ± 0.06	27.82 ± 0.07	27.84 ^{ef}
T ₇	SA@200ppm	4958 ± 8.32	4985 ± 8.42	4971 ^m	27.78 ± 0.08	27.95 ± 0.01	27.87 ^{de}
T ₈	HG@3.75kg/ha+ SA@100ppm	5469 ± 7.67	5508 ± 11.29	5489 ^d	27.74 ± 0.02	28.00 ± 0.02	27.87 ^{de}
T ₉	HG@3.75kg/ha+ SA@200ppm	5412 ± 8.42	5561 ± 7.27	5487 ^{de}	28.18 ± 0.09	28.01 ± 0.02	28.10 ^{bcd}
T ₁₀	HG@5kg/ha+ SA@100ppm	5487 ± 6.99	5616 ± 8.36	5552 ^b	28.05 ± 0.09	27.95 ± 0.04	28.00 ^{cde}
T ₁₁	HG@5kg/ha+ SA@200ppm	5518 ± 7.81	5601 ± 8.42	5559 ^a	28.24 ± 0.05	28.18 ± 0.07	28.21 ^{bc}
T ₁₂	GK@15kg/ha+ SA@100ppm	5199 ± 10.93	5272 ± 8.03	5235 ⁱ	28.08 ± 0.07	27.97 ± 0.04	28.02 ^{bcd}
T ₁₃	GK@15kg/ha+ SA@200ppm	5268 ± 8.94	5317 ± 9.86	5292 ^h	28.02 ± 0.03	28.13 ± 0.01	28.07 ^{bcd}
T ₁₄	GK@20kg/ha+ SA@100ppm	5317 ± 9.30	5379 ± 7.16	5353 ^g	28.00 ± 0.32	27.98 ± 0.05	27.99 ^{cde}
T ₁₅	GK@20kg/ha+ SA@200ppm	5327 ± 8.08	5451 ± 6.45	5389	28.10 ± 0.07	28.00 ± 0.02	28.05 ^{bcd}
T ₁₆	Irrigated(IR)	5435 ± 8.61	5601 ± 9.86	5518 ^c	29.91 ± 0.03	30.08 ± 0.04	30.02 ^a
Mean		5195	5284	5240	28.17	28.14	28.15
SE(m)		1.89	2.14	2.53	0.09	0.03	0.08
CD at 5% level of significance		4.49	5.21	4.33	0.28	0.11	0.24
CV (%)		0.06	0.07	0.07	0.35	0.22	0.40

**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

**Treatments with the same letters are not significantly different

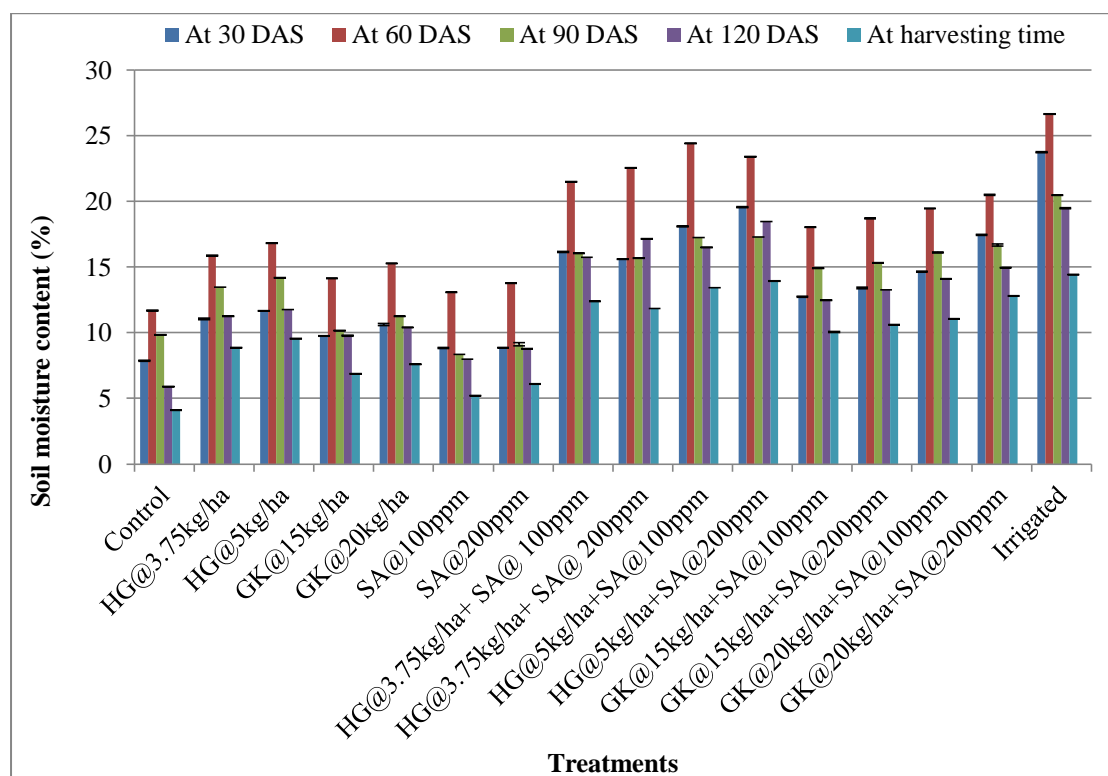
4.6.10 Harvest index (%)

Upon examination of the data presented in Table 15, it is evident that there was a significant decrease in harvest index under rainfed conditions in comparison to irrigated treatments. However, the implementation of gond katira@20kg/ha demonstrated a marked improvement in harvest index, surpassing the effects of gond katira@15kg/ha and the control treatment. The lowest harvest index was observed in the rainfed treatment, with values ranging from 27.60 to 30.02%. The greatest reduction in harvest index, at 8.6%, occurred in the control (rainfed) group, which was mitigated by 2.3% through the application of gond katira@20kg/ha and gond katira@15kg/ha, respectively.

4.7 Soil parameters

4.7.1 Soil moisture

The soil moisture data were recorded at various intervals throughout the crop cycle, including at 30, 60, 90, and 120 days after sowing, as well as at the time of harvesting. This data was collected at different depths, ranging from 0-15cm, 15-30cm, and 30-60cm, and can be found in Figure 20.



**HG- Hydrogel; GK- Gond Katira; SA- Salicylic acid

Figure 20: Effect of salicylic acid and hydrogel treatments on soil moisture content (%) of Indian mustard variety RH 725 under rainfed conditions (Values are means of two Rabi seasons (2019-20 and 2020-21) and vertical bars indicate standard error (\pm))

Upon analyzing the data presented in Figure 20, it is evident that rainfed conditions led to a significant decrease in soil moisture compared to irrigated treatment. However, when the hydrogel was applied at a rate of 5kg/ha with two foliar sprays of 200 ppm and 100 ppm salicylic acid at flowering and siliqua formation stages, there was a significant improvement in soil moisture. When compared to their corresponding treatments, the rainfed treatment recorded the lowest soil moisture levels at various intervals. At 30 DAS, soil moisture content ranged from 7.8 % to 23.7 %, while at 60 DAS, it ranged from 11.6 % to 26.6 %. At 90 DAS, it ranged from 12.8 % to 26.4 %, and at 120 DAS, it ranged from 5.8% to 19.4%. At harvesting time, soil moisture content was recorded at 4 % to 14.4 %. The control (rainfed) condition induced the maximum reduction in soil moisture at 10.4 %, with hydrogel@5kg/ha+SA (200 ppm) and hydrogel@5kg/ha+SA (100 ppm) alleviating these reductions by 3.9 % and 3.4 %, respectively. The results showed that GK treatments also improved the soil moisture content when compared with the control conditions but GK treatments are not retained much soil moisture as irrigated and hydrogel treatments.

The growing world population is currently suffering from global food shortages caused by increased processes leading to increased global warming (Lesk *et al.*, 2016; Chakraborty *et al.*, 2018). Abiotic stresses significantly affect physiological processes, resulting in altered metabolic activity and vigorous plant growth (Dwivedi *et al.*, 2020). Global warming makes drought a major abiotic challenge to crop production, productivity and food security. It also leads to erratic weather patterns and reduced rainfall, leading to frequent outbreaks of drought around the world (Lobell *et al.*, 2011). Subsequent drought events significantly affect plant growth and yield, arrest their development, affect their physiology and prevent their reproduction (Yordanov *et al.*, 2000).

Mustard greens respond better to irrigated conditions as compared to rainfed conditions due to low winter rainfall. This crop is more sensitive to moisture fluctuations and is more or less in a critical growth phase, which has a negative impact on yield (Meena *et al.*, 2013). Irrigation during the critical period increased yield in Indian mustard, probably due to better nutrient availability and more efficient metabolic activity of the plant (Mehta, 2004). Furthermore, it is generally accepted that limited irrigation water can be used more efficiently by scheduling irrigation during critical crop growth phases.

The findings and key results of this study entitled "**Enhancing physiological efficiency and yield potential of Indian mustard using hydrogels and salicylic acid under rainfed conditions**" are discussed below under the following headings:

- 5.1 Growth and reproductive parameters
- 5.2 Physiological parameters
- 5.3 Biochemical parameters
- 5.4 Membrane injury indices
- 5.5 Antioxidant defence system
- 5.6 Yield and yield attributing characters
- 5.7 Soil parameters

5.1 Growth and reproductive parameters:

Growth and reproductive parameters are the best indicators for assessing plant responses to environmental stress. Our data suggest that drought damages plant growth and reproductive traits. This experiment investigated the effects of salicylic acid and hydrogel treatments on the morphology and reproductive traits of Brassica cultivar RH 725 under rainfed stress. The application of hydrogels and salicylic acid resulted in improved growth and

reproductive parameters of Brassica plants under rainfed conditions (Figures 3, 4 and 5). However, hydrogel application with two foliar sprays of salicylic acid during the flowering and siliqua formation stage was the most effective treatment in the induction of growth parameters of Brassica plants, under rainfed conditions. In the case of rainfed, the Application of hydrogel at 5 kg/ha + SA (200 ppm) and hydrogel at 5 kg/ha + SA (100 ppm) resulted in an average increase in plant height, flowering time and days to physiological maturity as compared to rainfed and all other treatments. The reduction in these attributes during rainfed conditions is likely due to instability in the functioning of stomata and root architecture, which impede the supply of water and nutrients needed for proper metabolic processes in plants. This phenomenon has been observed and studied by Kosar *et al.* (2015); Anjum *et al.* (2017) and Hussain *et al.* (2019).

In any crop, the most critical stage that can be impacted by abiotic stress is reproduction. The occurrence of water stress days with greater frequency during flower development has an impact on plant reproduction that is immediate and long-lasting, as evidenced by Kartikeya *et al.* (2012). Drought stress generally leads to early flowering, and in the current study, the initiation of flowering occurred three days earlier in the rainfed treatment (Figure 3).

The early flowering of plants in drought conditions may be attributed to their rapid phenological development, which enables them to complete their life cycle despite the adverse environment. In agriculture, super-absorbent polymers are commonly utilized as water-retaining substances. When incorporated into the soil, these polymers can store significant amounts of water and nutrients, which are gradually released to support crop growth in the face of limited water supply (Yazdani *et al.*, 2007). According to research conducted by Yazdani *et al.* (2008), the impact of superabsorbent polymer and water stress on the qualitative and quantitative performance of soybean seed protein was the most significant. Additionally, Orikiroza *et al.* (2009) suggested that adding hydrogel amendment to soil enhances the efficiency of water uptake and utilization of photosynthetic plants, especially those grown in soil with water contents near field capacity.

Agaba *et al.* (2010) showed that hydrogels enhanced plant growth and significantly increased shoot and root biomass compared to controls. Barihi *et al.* (2013) observed that the growth index of cucumbers in greenhouses can be improved by using superabsorbent polymers. Waly *et al.* (2015) reported that 1% hydrogels excelled in all growth and yield traits in dried rice, producing the tallest plants and the most shoots.

Plant growth and development of oilseeds were improved by imposing hydrogel-mediated photosynthesis, higher soil moisture and optimal nutrient transfer under limited irrigation and rainfall conditions (Sivapalan 2011; Rathore *et al.*, 2019). Nutrient availability increased through prolonged availability of water and uptake improved plant growth and yield

characteristics in hydrogel-modified plots (Chen *et al.*, 2003; Rathore *et al.*, 2019).

Exogenous application of salicylic acid reduced the negative effects of water stress (Khan *et al.*, 2015; Meena *et al.*, 2018), and spraying salicylic acid improved plant growth (Hayat *et al.*, 2010). Salicylic acid plays a key role in providing tolerance to plants undergoing water stress, viz. drought or flood (Hayat *et al.*, 2010). Exogenous application of salicylic acid promotes growth; and flowering; upregulates photosynthesis; and increases the activity of enzymatic and non-enzymatic antioxidants (Arif *et al.*, 2020).

5.2 Physiological parameters:

Photosynthesis, as well as transpiration rate and stomatal conductance, decreased at both sampling stages (flowering and siliqua formation stage) under rainfed stress, while hydrogel and foliar sprays of salicylic acid were applied twice during flowering and siliqua formation stages, these parameters were significantly improved compared to the control (RF) (Tables 1, 2 and 3). In the past, several researchers have documented that drought-induced stomatal limitation alters plant metabolic pathways by reducing CO₂ and nutrient uptake (Fathi and Tari, 2016; Hussain *et al.*, 2018; Hussain *et al.*, 2019). The first physiological response of plants under the influence of drought stress is to reduce transpiration through stomata (Hartmann *et al.*, 2013). Closing stomata and reducing plant water loss are physiological responses to avoid drought (Chaves *et al.*, 2009; Murata and Mori, 2013). On the other hand, the closure of stomata affects physiological and biochemical processes, e.g. leaf water content, chlorophyll amount, chloroplast fragmentation, gas interactions, root-shoot ion exchange, and photosynthesis are reduced, while morphologically inhibited leaf expansion (Bray, 2002; Mumm *et al.*, 2011; Rollins *et al.*, 2013; Potopova *et al.*, 2016; Fahad *et al.*, 2017;). As a result, all these processes and physiological events directly or indirectly affect photosynthetic activity (Zhang 2007; Muhammad *et al.*, 2021; Sharma *et al.*, 2019). Plants control the flow of air and water through stomata in their leaf tissue. Stomatal closure due to drought prevents the use of carbon dioxide, which is important for photosynthesis (Sevanto, 2014). Reduced uptake of CO₂ by plants leads directly to reduce photosynthetic activity (Flexas *et al.*, 2004). Stomatal closure during water scarcity reduces transpiration, which also limits the uptake of nutrients from the soil by the roots and their transfer to the upper part of the plant (Kheradmand *et al.*, 2014). This situation leads to a dramatic decrease in plant tissue nutrient concentrations and ion balance (Kheradmand *et al.*, 2014; Ahanger *et al.*, 2016). Many processes are affected by the disrupted flow of nutrients, minerals, water and gases in plant tissues (Bhargava and Sawent, 2013; Ying *et al.*, 2013; Rivas *et al.*, 2016).

Relative water content is another important physiological trait that affects leaf water potential, stomatal resistance, transpiration rate, and plant water condition (Hartmann *et al.*, 2013). Relative water content is considered an indicator of plant water status, which regulates metabolic activity in tissues. Relative water content is a result of water loss due to transpiration and root uptake (Nayyar and Gupta, 2006; Georgii *et al.*, 2017). Leaf water potential, which is

important for plant survival and photosynthetic processes; turgor pressure is closely related to stomatal closure and cell growth (Sun *et al.*, 2013; Alghabari *et al.*, 2015). Low to moderate water stress is tolerated by maintaining leaf water potential. However, the reduction in photosynthetic efficiency is due to increased water stress leading to increased potential leaf water loss (Nikinmaa *et al.*, 2013). Application of superabsorbent polymers improves cell membrane development, leaf area index, leaf area durability, chlorophyll and protein content by balancing nutrients, and leads to increased yield traits in mustard greens through longer stomata opening for higher CO₂ fixation (Dexter and Miyamoto, 1995; Rathore *et al.*, 2019). Continuing with the gas exchange parameters discussed above, there is a good correlation between transpiration rate, stomatal conductance, relative water content, and plant water status (Gama *et al.*, 2009; Vysotskaya *et al.*, 2010). The present findings suggest that drought stress affects the water condition of plants due to reduced or no water availability, i.e. RWC, Ψ_w and Ψ_s for the two sampling stages (flowering and silique formation stage), but the trend reversed with the application of hydrogel and foliar sprayed salicylic acid (Tables 4, 5 and 6). The reduction in water availability leads to further reductions in leaf water potential and turgor potential (Alferez *et al.*, 2010). Manivannan *et al.*, (2007) and Hussain *et al.*, (2019) documented a significant reduction in photosynthetic pigments in sunflowers under drought stress due to excessive ROS production, insufficient plant nutrient uptake, and disruption of cellular enzyme activity. Stomatal closure is a typical response to dehydration, often positive or negative, and may result in shoot or root dehydration (Chaves *et al.*, 2009). Hajiboland *et al.* (2017) showed that moderate drought resulted in a significant decrease in the stomatal opening, which was closely associated with a decrease in leaf and root osmotic potential and leaf-relative water content. Stomatal closure is controlled by chemical cues, primarily abscisic acid, which is produced by root dehydration when the dehydration status of leaves is unchanged (Farooq *et al.*, 2009; Wilkinson and Davies, 2010). The present results showed that hydrogel applications with two foliar sprays of salicylic acid during the flowering and silique formation stages of Brassica plants significantly increased the water relationship of the plants under rainfed conditions. According to Rathore *et al.* (2020), the highest relative water content was recorded for hydrogels with an irrigation water/cumulative pan evaporation (IW/CPE) ratio of 0.8 during the pre-anthesis and pod development stages in wheat crops. Similar RWC with scheduled irrigation of 0.8 IW/CPE-HG (without hydrogel) and 0.6 IW/CPE + HG (with hydrogel) indicated that the hydrogel response was poorer at higher irrigation regimes. Among other irrigation systems, hydrogels significantly improved relative water content. Relative water content at the pod development stage had a significantly higher impact on seed yield than at the pre-anthesis stage. The relative water content of leaves irrigated with 0.6 IW/CPE-HG hydrogel was at the same level as that of 0.4 IW/CPE+HG. Likewise, rainfed plants +HG and plants irrigated with 0.4 IW/CPE -HG showed similar leaf-relative water content (Rathore *et al.*, 2020). Drought stress

decreased chlorophyll fluorescence, chlorophyll index and chlorophyll stability index at two sampling stages (flowering and siliqua formation stage), but after hydrogel applications with spraying salicylic acid twice on the leaves at flowering and pod formation stage, increased these parameters as compared with the control (rainfed) plants (Table 7 and 8) (Figure 6). Under rainfed conditions, maximum chlorophyll fluorescence, chlorophyll index and CSI were observed in hydrogel@5kg/ha+SA(200ppm) and hydrogel@5kg/ha+SA(100ppm), showing positive effects of hydrogel and salicylic acid (Table 7 and 8) (Figure 6). Recently, it has never been more important to produce more food and avoid crop loss to meet the demands of a growing population. However, a large portion of cultivated land is affected by abiotic stresses (drought, salinity, cold, high temperature, heavy metal toxicity, ultraviolet radiation, etc.), which are expected to increase due to climate change, further exacerbating the occurrence of anthropogenic activities. These abiotic stresses lead to changes in plant physiological and biochemical processes, resulting in reduced plant growth and yield (Wani and Sah, 2014). These stresses lead to rapid changes in cellular redox homeostasis and excessive formation of reactive oxygen species (ROS), ultimately damaging organelles and interfering with ROS-promoted signalling pathways (Noctor *et al.*, 2018). In contrast to the overproduction of ROS, a physiological redox state impedes normal cellular function and impairs the plant immune system, suggesting that a threshold level of ROS is required for normal plant function (Farooq *et al.*, 2019). Flavonol biosynthesis and accumulation in plants are also stimulated under water-deficient conditions, along with increased resistance to drought stress (Kirakosyan *et al.*, 2003; Ballizany *et al.*, 2012). Drought stress also modulated the biosynthetic pathways of phenolic acids and flavonoids, leading to increased accumulation of these compounds (Li *et al.*, 2018; Rezayian *et al.*, 2018), which act as antioxidants and protect plants from water stress Negative effects of (Nichols *et al.*, 2015). For example, increased levels of flavonoids such as kaempferol and quercetin in tomato plants have been associated with increased drought tolerance (Sánchez-Rodríguez *et al.*, 2011). Harmful H₂O₂ molecules induced by drought stress can be effectively detoxified by accumulating flavonoids in the cytoplasm. Finally, flavonoid oxidation is followed by ascorbate-mediated reconversion of flavonoids to primary metabolites (Hernández *et al.*, 2009).

Flavonoids, including anthocyanins, are potent antioxidants (Yamasaki *et al.*, 1997; Yamasaki, 1997), but are spatially separated from the sites of oxidant production in chloroplasts and mitochondria. Despite severe quenching in these organelles, H₂O₂ leaks into the vacuole under severe pressure. Yamasaki (1997) suggested that H₂O₂ is quenched by anthocyanins and other phenolics. However, the equivalent potency of other colourless flavonoids and phenolics as antioxidants suggest that the putative photooxidative protection provided by anthocyanins is independent of their ability to scavenge oxidants. Instead, it is conceivable that anthocyanins protect plants from photooxidation by attenuating visible light

and the resulting reduction in excitation stress (Smillie and Hetherington, 1999; Merzlyak and Chivkunova, 2000; Feild *et al.*, 2001). In our study, data showed that flavonols and anthocyanins accumulate at a higher rate in both sampling stages (flowering and silique formation) under rainfed conditions. However, minimum anthocyanin and flavonol index were observed in hydrogel@5kg/ha+SA(200ppm) and hydrogel@5kg/ha+SA(100ppm), showing positive effects of hydrogel and salicylic acid as compared to rainfed condition (Table 9 and 10).

5.3 Biochemical parameters: Organic compounds such as proline and glycine betaine are known osmoprotectants (Ashraf and Harris, 2004). Genard *et al.* (1991) reported that glycine betaine delayed the loss of photosynthetic activity during storage in isolated chloroplasts by inhibiting chlorophyll degradation. Glycine betaine protects physiological processes such as photosynthesis and protein synthesis during drought (Sulpice *et al.*, 1998). Furthermore, these osmolytes help maintain osmoregulation, thereby maintaining swelling and avoiding osmotic pressure (Ashraf and Foolad, 2007). Our study showed that proline and glycine betaine contents were significantly increased under rainfed conditions (Figures 7 and 8). The increase of osmolytes in plant cells may be due to various responses such as ROS detoxification, membrane integrity, the stable activity of various enzymes, and osmotic adaptation to induce plant drought tolerance. Wu *et al.*, (2015) reported the accumulation of proline in drought-tolerant cotton plants exhibiting osmotic adaptation under drought stress. However, Pei *et al.*, (2010) argued that increased accumulation of proline and glycine betaine indicated stress-induced impairment rather than their role in stress tolerance. Soluble sugar accumulation in plants under drought stress plays a key role in stress mitigation through ROS scavenging, carbon storage, and osmoprotectants (Gupta and Huang, 2014). In this study, the application of hydrogel and two foliar sprays of salicylic acid during flowering and pod formation reduced osmolyte accumulation in leaf tissues of Brassica plants under stress conditions. Under rainfed conditions, hydrogel at a dose of hydrogel@5kg/ha+SA(200ppm) and hydrogel@5kg/ha+SA(100ppm) significantly decreased the proline, glycine betaine and TSS accumulation as compared to the control (rainfed). A minimum increase in the proline content under rainfed conditions was observed in hydrogel@5kg/ha+SA(200ppm)(6.1%) preceded by hydrogel@5kg/ha+SA(100ppm)(12%) over irrigated conditions ones coupled with a minimum increase of the glycine betaine content under rainfed conditions was observed in hydrogel@5kg/ha+SA(100ppm)(12%) preceded by hydrogel@5kg/ha+SA(200ppm)(24.2%) over irrigated conditions (Figure 7 and 8). The same trend was followed in the case of total soluble sugars (Figure 9). The decrease in proline, glycine betaine and TSS accumulation under drought stress on the application of hydrogel and salicylic acid gives us an indication of stress release and alleviation of stress damage.

Drought stress was found to induce hydrogen peroxide, relative stress damage, and MDA content in Brassica (Figures 10, 11 and 12). The overproduction of ROS under low water stress leads to the massive accumulation of these biochemicals. The increase in H₂O₂ suggests that oxidants other than these may be produced, which contribute to the increase in H₂O₂ levels under drought stress (Moller *et al.*, 2007).

The process of stomatal closure and increased flux through the photorespiratory pathway increases tissue oxidative stress because both processes generate reactive oxygen species (ROS), especially hydrogen peroxide (H₂O₂). Hydrogen peroxide is also produced as a second messenger in abscisic acid (ABA)-mediated stomatal closure (Pei *et al.*, 2000). During photorespiration, the glycolate oxidase reaction in the peroxisomes produces H₂O₂ at very high rates (Noctor *et al.*, 2002). In our study, the decrease in H₂O₂ accumulation under drought stress on the application of hydrogel and salicylic acid gives us an indication of stress release and alleviation of stress damage.

5.4 Membrane injury indices

Drought stress resulted in decreased membrane stability index (MSI) and increased membrane injury (MI). The reduction in MSI was greater in plants under severe stress than in moderate stress treatments and was more pronounced at the onset of stress than at the tillering stage. Tolerant plants maintained a significantly higher MSI during stress and exhibited lower MI compared to responsive varieties (Abid *et al.*, 2018).

MDA is a marker of oxidative damage in plants under stress conditions (Hussain *et al.*, 2016). MDA was used as a marker/signal to detect lipid peroxidation under stress conditions and the accumulation of ROS leading to membrane instability reflecting lipid peroxidation. In addition, due to the formation of free radicals such as superoxide radical (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radical (OH[•]) formed under drought stress, the cell membrane is damaged, and the degradation of nucleic acid and protein is leading cause of injury oxidation (Sairam *et al.*, 2000; Neill *et al.*, 2002; Moller *et al.*, 2007; Nair *et al.*, 2008 and Hussain *et al.*, 2018). There are numerous reports in the literature that drought reduces MDA and H₂O₂ in wheat (Kosar *et al.*, 2015), cucumber (Ma *et al.*, 2004) and rapeseed (Akram *et al.*, 2018) under rainfed conditions. This study showed that under drought stress, hydrogels application with salicylic acid sprayed twice foliarly during the flowering and pod formation phase significantly reduced H₂O₂, lipid peroxidation and RSI at both sampling stages (flowering and siliqua formation) (Figures 10, 11 and 12). This decrease is an indication of a stress damage mitigation strategy. The treatments with hydrogel@5kg/ha+SA(200ppm) resulted in a lower accumulation of ROS in the water-stressed plants which in turn decreased the MDA levels in the leaves.

5.4 Antioxidant defence systems:

Water stress leads to the formation of reactive oxygen species, which are extremely harmful to plants. In response to damage caused by ROS, plants have evolved an antioxidant defence system that includes enzymes such as SOD, CAT, APX, POX, and GR (Gratao *et al.*, 2008). Furthermore, SOD is the first line of defence against ROS, responsible for the conversion of O_2^- to H_2O_2 , which is further detoxified by POD, CAT, and APX (Moller *et al.*, 2007). SOD is one of the most important antioxidant enzymes capable of controlling oxidative damage caused by ROS. Drought stress increases the level of superoxide radicals (O_2^-) in cells. SOD catalyzes these free radicals to O_2 and H_2O_2 . If these free radicals are not intercepted by SOD, they can disrupt the activity of various biomolecules (Mittler, 2002). When SOD activity was high, superoxide radicals were properly scavenged, leading to increased drought tolerance. Under these conditions, increased SOD rapidly removes O_2^- , thereby reducing the risk of OH, thereby conferring stress tolerance on plants. In our study, SOD activity significantly increased with increasing leaf-rainfed stress in both sampling stages. In plant cells, there is an alternative and more efficient H_2O_2 detoxification mechanism that is active in both the chloroplast and the cytosol (Foyer and Nocotor, 2005). Plants respond to various environmental cues to survive stresses such as drought (Pastori and Foyer, 2002). Strategies to minimize oxidative damage are a universal feature of plant defence. Hydrogen peroxide is eliminated by enzymes catalase and ascorbate peroxidase (Chen and Asada, 1989; Scandalios *et al.*, 1997). These enzymes rapidly destroy most of the H_2O_2 produced by metabolism, but allow low steady-state levels to be maintained, presumably to maintain redox signalling pathways (Noctor and Foyer, 1998). Our studies showed that the activities of SOD, CAT and APX were stimulated under drought stress (Figures 13, 14 and 15). Several studies have reported increased stress tolerance associated with overstimulation of antioxidant enzyme activity (Sairam and Saxena, 2000; Sairam *et al.*, 2005; Tan *et al.*, 2006 and Zlatev *et al.*, 2006). In our investigation the specific activity of SOD, CAT and APX was increased to 48.8%, 80% and 49% under control (rainfed) conditions, respectively, which was further reduced by 45.8%, 73.6% and 44.6% with the application of hydrogel@5kg/ha+SA(200ppm) as compared to IR condition. This decrease is an indication of a stress damage mitigation strategy.

5.4.1 Ascorbate-Glutathione pathway

The ascorbate-glutathione pathway (also known as the Asada-Halliwell pathway) is a major antioxidant defence pathway that primarily detoxifies H_2O_2 in plant cells. In addition to AsA and GSH, its enzymes APX, MDHAR, DHAR and GR (Hasanuzzaman *et al.*, 2012) also play important roles. Both AsA and GSH are found in the cytosol, nucleus, chloroplasts, mitochondria, and peroxisomes, where they function with the support of four enzymes. Therefore, each enzyme has multiple isoforms based on cellular localization (Hasanuzzaman *et al.*, 2017). Both AsA and GSH are present in millimolar concentrations in organelles, for

example in Arabidopsis, AsA concentrations are highest in peroxisomes (22.8 mM) and GSH in mitochondria (14.9 mM) (Zechmann, 2014; Hasanuzzaman *et al.*, 2017). Both AsA and GSH have high redox potentials and thus interact with many components and pathways to maintain a generally reduced state. There are a few steps in which AsA and GSH detoxify H₂O₂ in a coordinated manner, and AsA and GSH are regenerated simultaneously. First, with the help of AsA as an electron donor, the APX enzyme converts H₂O₂ into water, which in turn is converted into monodehydroascorbic acid (MDHA). This MDHA regenerates AsA through the activity of MDHAR, a part of which is spontaneously converted into dehydroascorbic acid (DHA). Later, DHA is reduced to AsA by using GSH, causing its oxidation to GSSG. Finally, this GSSG uses NADPH as an electron donor to regenerate GSH through the activity of GR. Both AsA and GSH are powerful antioxidants, but maintaining their redox state is important for conferring stress tolerance in plants, which largely depends on the activities of four enzymes associated with the AsA-GSH cycle (Hasanuzzaman *et al.*, 2012; Szarka *et al.*, 2012). In our investigation, the oxidized ascorbate (DHA) and oxidized glutathione (GSSG) was decreased under control (rainfed) condition however, a hydrogel with two foliar sprays of salicylic acid at flowering and siliqua formation stage induced increases in the oxidized ascorbate (DHA) and oxidized glutathione (GSSG) were more prominent under rainfed conditions. Across treatments, hydrogel@5kg/ha+SA(200ppm) and hydrogel@5kg/ha+SA(100ppm) application averagely increased the oxidized ascorbate (DHA) and oxidized glutathione (GSSG) under rainfed conditions. This decrease is an indication of a stress damage mitigation strategy. In contrast to oxidized ascorbate (DHA) and oxidized glutathione (GSSG), reduced ascorbate (AsA) and reduced glutathione (GSH) were increased under rainfed conditions, which was reduced with the application of hydrogel@5kg/ha+SA(200ppm) and hydrogel@5kg/ha+SA(100ppm). This decrease in reduced ascorbate (AsA) and reduced glutathione (GSH) under drought stress on the application of hydrogel and salicylic acid gives us an indication of stress release and alleviation of stress damage (Figures 16, 17, 18 and 19).

5.5 Yield and yield attributing characters

The number of primary and secondary branches, main shoot length, number of pods per plant, number of seeds per pod, test weight, seed yield and biomass, straw yield and harvest index under rainfed conditions compared to observed irrigation conditions dropped significantly. But after the application of hydrogel and salicylic acid, the reduction rate in yield and its attributes decreased (Tables 11- 15). Yazdani *et al.* (2007) reported a significant increase in soybean seed yield (5495 kg ha⁻¹) after superabsorbent polymer application compared to the control (4172 kg ha⁻¹). Eiasu *et al.* (2007) found that pure gel polymers increased overall and marketable tuber yield, especially at high fertilization rates. Ghamsari *et al.* (2009) reported that the use of superabsorbent polymers helped alleviate drought stress and increase kernel number and kernel weight in maize.

SA is an important plant growth regulator. Kothule *et al.* (2003) also reported an increase in the number of branches of plant⁻¹ in soybean due to the application of PGRs. Plants had a lower number of branches observed under drought conditions compared to plants under normal conditions. Foliar spraying of PGRs improved the number of branches plant⁻¹ compared to the control, and a maximum and the significant increase in the number of branches plant⁻¹ was recorded at an SA of 0.7 mM. These results are consistent with Rao *et al.* (2009) who reported that SA minimized drought stress and improved branch number. Sadeghipour and Aghaei (2012) reported similar results for pulses. Treatment with SA 0.7 mM showed the most significant increase in the number of pods plant⁻¹ over the control, followed by SA 0.5 mM. The increase in the number of Siliquae plant⁻¹ may be due to better transfer of nutrients and assimilation to the reproductive area. These results are consistent with Akhter *et al.* (2007) in Mustard. Under drought conditions, the number of pods plant⁻¹ decreased, but the plant growth regulator increased the number of pods plant⁻¹. The maximum amount of Siliquae plant⁻¹ was recorded for SA 0.7 mM, followed by SA 0.5 mM. This finding is supported by previous findings on mustard by Singh and Usha (2003). Drought conditions adversely affected the number of seeds siliqua⁻¹, however, the application of PGR increased the number of seeds siliquae⁻¹ compared to the control. These results are supported by Singh and Usha (2003) in Mustard.

The impact of various irrigation schedules and hydrogel application on seed yield and yield attributes was examined by Bharat *et al.* (2019) in a study. The most successful seed yield and yield attributes, including 1000 seed weight, siliqua per plant, seeds/siliqua, and harvest index, were observed with irrigation scheduling among all irrigation scheduling treatments. This result may be attributed to the fact that irrigation scheduling at different intervals resulted in a notable effect on cumulative water use efficiency. Similar outcomes were reported by Singh *et al.* (2017). Conversely, plots without irrigation recorded the lowest seed yield and yield attributes.

According to Bharat *et al.* (2019), among different hydrogel treatments, 5 kg/ha hydrogel application, although equivalent to 2.5 kg/ha hydrogel application, resulted in a significant increase in seed yield and yield-related traits, i.e. 1000 seed weights, siliqua per plant, seeds/ siliqua and harvest index as a comparison to no hydrogel applied during the two experimental years. However, a significant increase was recorded in the hydrogel-applied plots that were not irrigated for both test years. Similar outcomes were reported by Kumar *et al.* (2016) and Singh *et al.* (2017). However, the lowest yield and yield-related traits were recorded in plots where no hydrogel was used. This may be due to periods of plentiful water supply i.e. resulting in the absorption/retention of moisture. The ability of the field to release water during water stress results in an increase in the potential energy of the soil matrix and provides sufficient moisture to the crop throughout the vegetation and reproductive periods, thereby increasing the crop's photosynthetic accumulation, leading to a significant increase in

seed yield of the Indian mustard crop in two test years characteristics. Similar observations were also recorded by Bharat *et al.* (2017). Enhancing root development and regulating plant growth by improving water retention capacity and soil cluster structure through hydrogels helps to achieve higher yields (Akelah, 2013). Singh *et al.* (2017) reported similar results in terms of yield advantage and higher nutrient uptake in mustard, (Rathore *et al.*, 2019) in pearl millet, and (Dai *et al.*, 2017) in sorghum.

5.7 Soil moisture content

Observation of data presented in Figure 20 clearly showed that soil moisture decreased significantly with rainfed conditions over irrigated treatment. Application of hydrogel@5kg/ha with two foliar sprays of 200ppm salicylic acid at flowering and siliqua formation stage significantly improved soil moisture which was chased by hydrogel@5kg/ha with two foliar sprays of 100ppm salicylic acid at flowering and siliqua formation stage as compared to control. The lowest soil moisture was recorded in rainfed treatment at various intervals as compared to their corresponding treatments.

Using hydrogels to increase the water storage capacity of soil can significantly reduce the watering needs of many plants, saving costs associated with watering. The overall B:C ratios suggest that higher economic benefits can be achieved by applying hydrogels under low irrigation conditions. Moisture remains trapped in the hydrogel-embedded soil due to reduced deep seepage and reduced evaporation, and surface runoff losses in sandy soils. They form three-dimensional expansive networks in compatible hydrous soils, store water and plant nutrients dissolved in the water, and gradually release water and nutrients as needed (Sharma 2004). Hydrogels are expected to reduce soil water tension by improving soil moisture under water stress conditions (Wang *et al.*, 2003). Lighter textured soils under semiarid and arid regions are poor in moisture and nutrients (Riofrio and Wittmeyer 1992; Li *et al.*, 2000), have large pores, rapid water loss, rapid decomposition of organic carbon, poor water retention away from nutrients, and often before plants absorb them for use. Adsorption of water and nutrients through inter- and intra-aggregate adhesion between soil particles and gels (Akhter *et al.*, 2004) activates favourable chemical reactions in the soil (El-Hady and Camilia, 2006). Hydrogels provide soil water reservoirs in the root zone by preventing leaching and deep leaching losses (Sow *et al.*, 1997). Higher retained porosity and lower saturated hydraulic conductivity under hydrogel-enhanced soils reduce drainage porosity (Paluszek and Zembrowski, 2008), thereby providing higher moisture content (Al-Darby, 1996; Al-Omran and Al-Harbi, 1998). The strong water storage and release properties of hydrogels allow sandy soils to store more water (Sivapalan, 2011), protect against temporary drought stress, and reduce the risk of overall crop failure (DE Boot, 1990; Langaroodi *et al.*, 2013).

CHAPTER-VI

SUMMARY AND CONCLUSION

The present investigation entitled “**Enhancing physiological efficiency and yield potential of Indian mustard using hydrogel and salicylic acid under rainfed condition**” was conducted at the Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar, India. The crop was grown in the Research Farm of Oilseeds Section during two successive *Rabi* seasons of 2019-20 and 2020-21 in randomized block design (RBD) with 3 replications in plots size of 9m² in each replication with the spacing of 30 cm between the rows and 10 cm plant to plant distance.

Seeds were mixed with different levels of hydrogel (3.75kg/ha and 5kg/ha) and gond katira (15kg/ha and 20kg/ha) according to respective treatment.

The crop was sprayed with different concentrations of salicylic acid (SA @ 100ppm and 200ppm) with the help of a manual sprayer at the flowering and siliqua formation stage in rainfed environmental conditions. For the preparation of different levels of salicylic acid (100ppm and 200ppm), the chemical was first dissolved in 100 µl ethanol and then mixed in water. The crop was sampled after five days of spray. Various growth and reproductive parameter, physio-biochemical, antioxidative defence system, yield and yield attributing characters, and soil moisture content was analysed using a standardised method at different crop growth stages.

The analysis and interpretation of results under moistures stress and the mitigation mechanism are presented and summarized below under the following heads:

- Our research has indicated that the growth and phenology of Indian mustard are negatively affected by rainfed conditions. Compared to irrigated conditions, the rainfed environment resulted in a significant decrease of 7.6% in days to 50% flowering, 13.6% in plant height, and 10.2% in days to physiological maturity. However, the application of hydrogel and salicylic acid had a positive impact on the growth and phenology of the crop under stressful conditions. By applying hydrogel at a rate of 5kg/ha and SA at 200ppm during the flowering and siliqua formation stage, there was a significant increase of 12.8% in plant height, 5.6% in days to 50% flowering, and 9.3% in days to physiological maturity. The results showed that the applications of GK along with SA during the flowering and siliqua formation stage resulted in a significant improvement in plant height, days to 50% flowering and days to physiological maturity when compared to the control conditions.

- The physiological parameters were observed to decrease under rainfed conditions. The photosynthetic rate, transpiration rate, stomatal conductance, RWC, water potential, osmotic potential, quantum yield, CSI, and chlorophyll index were decreased by 51.4%, 88.1%, 86%, 27.4%, 25.7%, 28.3%, 35.6%, 39.6%, and 49.3%, respectively. In contrast, the anthocyanin index and flavonol index were increased by 78.7% and 33.7%, respectively, when compared to irrigated conditions. However, the application of hydrogel @ 5kg/ha + SA(200ppm) during the flowering and silique formation stage resulted in a significant improvement in photosynthetic rate (9.4%), transpiration rate (10%), stomatal conductance (9.3%), RWC (24.7%), water potential (25.4%), osmotic potential (22%), quantum yield (28.6%), CSI (37%), and chlorophyll index (46.8%) respectively, with a decrease in anthocyanin index (69.6%) and flavonol index (31%), respectively, when compared to other corresponding treatments. The results showed that the applications of GK along with SA during the flowering and silique formation stage resulted in a significant improvement in photosynthetic rate, transpiration rate, stomatal conductance, RWC, water potential, osmotic potential, quantum yield, CSI, and chlorophyll index with a decrease in anthocyanin index and flavonol index when compared to the control conditions.
- Biochemical parameters were estimated in leaf samples at both sampling stages (flowering and silique formation stage) of crop growth. Several components, including proline (57.9%), glycine-betaine (82.3%), TSS (52.2%), H₂O₂ (36.8%), and MDA contents (47.2%), as well as RSI (45.9%), the specific activity of antioxidants (SOD (48.8%), CAT (80%), APX (49%)), reduced ascorbate (62.3%), and reduced glutathione (68.1%) contents, experienced an increase in levels due to an increase in rainfed conditions in comparison to irrigated ones. On the other hand, there was a decrease in oxidized ascorbate (4.2 fold) and oxidized glutathione (74.1%) contents. The application of hydrogel at a rate of 5kg/ha + SA(200ppm) during the flowering and silique formation stage led to a significant decrease in proline (51.8%), glycine-betaine (72.1%), TSS (48.7%), H₂O₂ (33.6%), and MDA contents (44.8%) as well as RSI (47%), the specific activity of antioxidants (SOD (45.8%), CAT (73.6%), APX (44.6%)), and reduced ascorbate (58.3%) and reduced glutathione (58.7%) contents. Meanwhile, there was an improvement in oxidized ascorbate (3.7 fold) and oxidized glutathione (66.5%) contents compared to other treatments. The results showed that the applications of GK along with SA during the flowering and silique formation stage had a significant impact on biochemical parameters when compared to the control conditions.
- Upon completion of the harvest, the yield and its corresponding character attributes were evaluated. These attributes included the number of primary branches per plant

(54.5%), the number of secondary branches per plant (45.7%), the length of the main shoot (29.2%), the number of siliqua per plant (91.2%), the number of seeds per siliqua (49.2%), the weight of 1000 seeds (26%), the yield of seeds (26.2%), the biological yield (16.3%), the stover yield (12.4%), and the harvest index (8.6%). The data revealed that the yield attributes decreased substantially with increased dependence on rainfall in comparison to irrigated crops. However, the application of hydrogel at a rate of 5kg/ha combined with SA (200ppm) during the flowering and siliqua formation stage led to a significant increase the number of primary branches per plant (43%), the number of secondary branches per plant (23.7%), the length of the main shoot (23.5%), the number of siliqua per plant (82.7%), the number of seeds per siliqua (32.2%), the weight of 1000 seeds (15.2%), the yield of seeds (15.4%), the biological yield (14.2%), the stover yield (13.3%), and the harvest index (2.3%) respectively, when compared to other treatments. The results showed that the applications of GK along with SA during the flowering and siliqua formation stage resulted in a significant improvement in the yield and yield attributes when compared to the control conditions.

- Under rainfed conditions, the moisture content of the soil tends to decrease. When the soil's moisture content becomes depleted, it can cause a reduction in the plant's ability to intake water, leading to the closure of stomata and a decrease in the transpiration rate. Ultimately, this can result in a low accumulation of dry matter and reduced yield. However, when hydrogel at a rate of 5kg/ha is applied along with SA (200ppm) at flowering and siliqua formation stage over rainfed, higher soil moisture levels are maintained at all soil depths, including 0-15, 15-30, and 30-60 cm. The results showed that the applications of GK along with SA during the flowering and siliqua formation stage maintained higher soil moisture when compared to the control conditions.

After conducting careful observations, it can be concluded that Indian mustard variety RH 725 experiences a decrease in all morpho-physiological, biochemical, soil moisture content, yield and yield attributes under rainfed conditions. However, the contents of proline, glycine-betaine, TSS, H₂O₂, MDA, RSI(%), specific activity of antioxidants (SOD, CAT, APX), reduced ascorbate, and reduced glutathione contents have all increased. To alleviate the negative impact of rainfed stress on Indian mustard variety RH 725, the application of hydrogel @ 5kg/ha + SA (200ppm), hydrogel @ 5kg/ha + SA (100ppm), and GK @ 20kg/ha + SA (200ppm) during flowering and siliqua formation stages have shown better results in terms of morpho-physiological, biochemical, yield and yield attributes, and soil moisture content. Among all treatments, hydrogel @ 5kg/ha + SA (200ppm) applied during the flowering and siliqua formation stage proved to be the most effective in mitigating the effects of rainfed conditions on Indian mustard variety RH 725.

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ABSTRACT

Title of Thesis	Enhancing physiological efficiency and yield potential of indian mustard using hydrogel and salicylic acid under rainfed condition
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The present investigation entitled “**Enhancing physiological efficiency and yield potential of indian mustard using hydrogel and salicylic acid under rainfed condition**” was conducted at the Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar, India. The crop was grown in the Research Farm of Oilseeds Section, CCS Haryana Agricultural University, Hisar, during two successive *Rabi* seasons of 2019-20 and 2020-21 which is situated at a latitude of 29° 10'N and longitude 75° 46' E and altitude 215.2 m above main water level and falls within the semitropical region of Western zone of India. A field experiment was conducted to judge the effective dose of hydrogel, gongon and salicylic acid for increasing the physiological processes, productivity, quality, and water-use efficiency of Indian mustard in rainfed conditions. The experiment consisted of 16 treatment combinations. Among these treatments, the application of hydrogel @ 5kg/ha + SA (200ppm) at the flowering and silique formation stage had a positive impact on the physiological processes of the crop under rainfed conditions. The data was recorded for growth, physiological, biochemical and yield parameters, which had a positive impact on the application of hydrogel and salicylic acid under rainfed conditions. The growth and phenology of Indian mustard are negatively affected by rainfed conditions. By applying hydrogel at a rate of 5kg/ha and SA at 200ppm during the flowering and silique formation stage, there was a significant increase of 12.8% in plant height, 5.6% in days to 50% flowering, and 9.3% in days to physiological maturity. The photosynthetic rate, transpiration rate, stomatal conductance, RWC, water potential, osmotic potential, quantum yield, CSI, and chlorophyll index were decreased by 51.4%, 88.1%, 86%, 27.4%, 25.7%, 28.3%, 35.6%, 39.6%, and 49.3%, respectively. In contrast, the anthocyanin index and flavonol index were increased by 78.7% and 33.7%, respectively, when compared to irrigated conditions. However, the application of hydrogel @ 5kg/ha + SA(200ppm) during the flowering and silique formation stage resulted in a significant improvement in photosynthetic rate, transpiration rate, stomatal conductance, RWC, water potential, osmotic potential, quantum yield, CSI, and chlorophyll index by 9.4%, 10%, 9.3%, 24.7%, 25.4%, 22%, 28.6%, 37%, 46.8%, respectively, with a decrease in anthocyanin index and flavonol index by 69.6% and 31%, respectively, when compared to other corresponding treatments. These results were supported by the biochemical results viz. H₂O₂, MDA, RSI, TSS, proline, glycine betaine, reduced ascorbate, and reduced glutathione content, antioxidant enzyme activities like SOD, CAT, and APX that decreased in treated plants as compared to their respective control. Meanwhile, there was an improvement in oxidized ascorbate and oxidized glutathione contents after applying hydrogel and salicylic acid which may be the contributing factors for yield increment. Yield attributes include the number of primary and secondary branches, main shoot length, the number of silique, seeds per silique, 1000 seed weight, seed yield, biological yield, stover yield and harvest index decreases due to unavailability of water in the soil under normal conditions. However, the application of hydrogel at a rate of 5kg/ha combined with SA (200ppm) during the flowering and silique formation stage led to a significant increase in yield attributes by 43%, 23.7%, 23.5%, 82.7%, 32.2%, 15.2%, 15.4%, 14.2%, 13.3%, and 2.3% respectively, when compared to control treatment. When hydrogel at a rate of 5kg/ha is applied along with SA (200ppm) at flowering and silique formation stage over rainfed, higher soil moisture levels are maintained at all soil depths, including 0-15, 15-30, and 30-60 cm. From our results, it is concluded that hydrogel at a rate of 5kg/ha is applied along with SA (200ppm) at flowering and silique formation stage results in higher yields, with better physiological efficiencies as this concentration maintains the best plant water relations and source to sink strength which is self-explanatory in our results.

MAJOR ADVISOR

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Degree	University/Board	Year of Passing	Percentage	Subjects
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B.Sc. (Medical)	Govt. P.G. college, Hisar	2015	76.1%	Botany, Zoology and Chemistry
M.Sc. (Botany)	Kurukshetra University, Kurukshetra	2017	76.5%	All relevant subjects
NET-JRF	CSIR-UGC	2018	-	Life sciences

j) Co-curricular Activities	<p>Poster presentation in National Conference “Biodiversity & Environmental Sustainability in Modern Era” held on Feb. 16, 2019 at CRM Jat College, Hisar.</p> <p>2nd Position in poster presentation in National Seminar “Eco-Friendly Techniques for Sustainable Development” held on Feb. 20, 2019 at St. Wilfred’s P.G. College, Jaipur.</p> <p>Poster presentation in the theme “Innovation Challenges in Interdisciplinary Research & Practices” held on Mar. 24-25, 2022 at Sri Guru Granth Sahib World University, Fatehgarh Sahib.</p> <p>Online poster presentation in International Conference “Biotechnology Emergence in Science and Technology” held on Dec. 22-24, 2022 at SRM Institute of Science and Technology, Ramapuram Campus, Chennai. Participate in the “Hands-On Training Program on Basic and Advanced Analytical Instrumentation in Plant Physiology” organized by Department of Botany and Plant Physiology in association with spectra Agritec, New Delhi, COBS&H, CCS HAU, Hisar.</p>
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List of Publications (related to thesis work only): 4 (Abstract)

1. **Rani, N.** and Kumar, N. (2022). Efficacy of hydrogel and salicylic acid for enhancing yield potential of Indian mustard under rainfed condition. Abstract published in international conference on “Recent Trends in Science, Technology, Management &

- Humanities” on Mar. 29, 2022 at Jagannath University, Bahadurgarh. pp. 163-164.
2. **Rani, N.** and Kumar, N. (2022). Effect of hydrogel and salicylic acid on physiological efficiency and yield attributes of Indian mustard under moisture stress condition. Abstract published in VIIth international conference on “Global Research Initiatives for Sustainable Agriculture & Allied Sciences” on November 21-23, 2022 at Birsa Agricultural University, Ranchi. pp. 197-198.
 3. **Rani, N.** and Kumar, N. (2022). Indian mustard yield (*Brassica juncea*L.) in response to salicylic acid and herbal hydrogel (gond katira) under rainfed conditions. Abstract published in 4th international conference on “Environment and Society (ICES 2022)” on Dec. 23-24,2022 at Jiwaji University, Gwalior. pp.25.
 4. **Rani, N.** and Kumar, N. (2023).Indian mustard (*Brassica juncea*L.) yield and physiological efficiency in response to salicylic acid and SAP hydrogel under restricted conditions. Abstract published in international conference on “Climate Resilient Agriculture for Food Security and Sustainability” on Feb. 17-19, 2023 at CCS Haryana Agricultural University, Hisar.Pp. 639.

Neelam Rani

UNDERTAKING OF THE COPYRIGHT

I, **Neelam Rani**, Admission No. **2018BS16D**, undertake that I give copyright to the **Chaudhary Charan Singh Haryana Agricultural University, Hisar** of my thesis entitled, **“Enhancing physiological efficiency and yield potential of Indian mustard using hydrogel and salicylic acid under rainfed condition”**.

I also undertake that, patents, if any, arising out of the research work conducted during the programme shall be filed by me only with due permission of the competent authority of **CCS HAU, Hisar**.

Signature of student