

# Challenges of climate change

## Omics-based biology of saffron plants and organic agricultural biotechnology for sustainable saffron production

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Kashmir valley is a major saffron (*Crocus sativus* Kashmirianus) growing area of the world, second only to Iran in terms of production. In Kashmir, saffron is grown on uplands (termed in the local language as “Karewas”), which are lacustrine deposits located at an altitude of 1585 to 1677 m above mean sea level (amsl), under temperate climatic conditions. Kashmir, despite being one of the oldest historical saffron-producing areas faces a rapid decline of saffron industry. Among many other factors responsible for decline of saffron industry the preponderance of erratic rainfalls and drought-like situation have become major challenges imposed by climate change. Saffron has a limited coverage area as it is grown as a ‘niche crop’ and is a recognized “geographical indication,” growing under a narrow microclimatic condition. As such it has become a victim of climate change effects, which has the potential of jeopardizing the livelihood of thousands of farmers and traders associated with it. The paper discusses the potential and actual impact of climate change process on saffron cultivation in Kashmir; and the biotechnological measures to address these issues.

### Niche Area of Saffron Cultivation in India

Climate change and environmental degradation have led to increased desertification and soil depletion. This kind of environmental degradation and the exhaustion of natural resources, such as forests and fertile agricultural land, constitute critical obstacles affecting sustainability of agriculture production systems, especially in ecologically fragile zones like the Himalayas. Much of the irrigation water in India and Pakistan originates from Himalayan glaciers. These glaciers are rapidly melting and their summer stream-flow may get significantly reduced within a few decades. Generally speaking, the most drought-vulnerable parts of our planet are the regions between 15° and 30° of the

northern and southern latitudes, and the localities on the lee side of mountains.<sup>1</sup> The state of Jammu and Kashmir (J&K) is located between 32°17' to 36°58' North (latitude) and 73°26' to 80°30' East (longitude), encompassing the Western Himalayas and the Karakorum mountains. One of the largest states of the Indian Union, J&K covers an area of 222 236 km<sup>2</sup> and includes, besides the famous Kashmir valley, the area of Jammu, Ladakh, Baltistan, Gligit, Hunza, and Nagar. Among these, the Kashmir valley represents one of the major saffron (*Crocus sativus* L.; Iridaceae) growing areas of the world. The period when saffron was introduced into Kashmir is not precisely known. Evidence from the oldest historical treatise of Kashmir *Rajatarangini*, written by Kalhana in the 12th century, indicates that saffron was present in Kashmir even before the reign of King Lalitaditya in 750 AD. Saffron is known as “Kum Kum” and “Kesar” in Sanskrit, and “Koung” in the Kashmiri language.<sup>2</sup> Even though successful attempts to grow saffron in other parts of J&K state like Kargil<sup>3</sup> or other areas of India such as Uttar Pradesh and Himachal Pradesh have been reported,<sup>4</sup> almost all saffron production is actually limited to Kashmir.

Saffron, originating from the Arabic word “Zafaran” meaning yellow, is a fascinating spice steeped in rich history.<sup>5</sup> Its secret stem from the dried red stigmas which accumulate large amounts of three glucosylated apocarotenoids,<sup>6</sup> namely crocin, picrocrocins, and safranal, which, among the more than 150 volatile and aroma yielding compounds, contribute to the color, bitter flavor, and aroma so typical of saffron. Saffron is a high value low volume spice that grows throughout Mediterranean Europe and Western Asia between 10° west and 80° east longitudes and 30 to 50° north latitudes.<sup>7</sup> The spice is used as flavoring and coloring agent in food and is a vital part of the dye, perfumery, and flavoring industries. Saffron also has countless biological properties like anticancer, antimutagenic, and antioxidant.<sup>8</sup> As a result, saffron fetches the highest price as a spice in the world, at approx. US\$ 1100–11 000/kg (<http://en.wikipedia.org/wiki/Saffron>), depending upon the country of its production.<sup>9</sup> Its production is typically favored in countries where labor is cheap, such as Iran and Azerbaijan, but is also produced in countries like Greece, Spain, Argentina, or the USA with global production exceeding 200 tonnes<sup>10</sup> and newer areas being brought under its cultivation, viz. China and Japan.

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In Kashmir, saffron is grown on uplands (termed in the local language as “Karewas”), which are lacustrine deposits located at an altitude of 1585 to 1677 meters above mean sea level (amsl), under temperate climatic conditions.<sup>11</sup> The soils are heavy textured with silty clay loam as the predominant texture in upper horizons and silty clay in lower horizons. These soils are alfisols and are well drained. The soils are calcareous in nature with average calcium carbonate and organic carbon contents of 4.61 and 0.35%, respectively. The soil is slightly alkaline with pH ranging from 6.3 to 8.3 and with electrical conductivity between 0.09 and 0.30 dsm<sup>-1</sup>.<sup>12-14</sup>

Kashmir, despite being one of the oldest historical saffron-producing areas, is now facing a rapid decline of saffron industry.<sup>15</sup> The total area under this crop in the State in 2012 is 3785 ha with an annual production of 11 t while almost one and half decade back in 1997 the area recorded was 5707 ha with an annual production of 15.95 t (Fig. 1). The lowest productivity of 1.57 kg ha<sup>-1</sup> was recorded in 2003–04 due to an acute drought from 1999–2003. According to the data available for 1990–96 the area of saffron cultivation in Kashmir varied in a narrow range of 4036 to 4496 ha, with more or less constant annual production (13.0–14.1 t) and productivity (2.90–3.21 kg ha<sup>-1</sup>).

There are many factors responsible for decline of saffron industry in Kashmir viz., the lack of availability of good-quality corms as seed material, poor soil fertility, lack of assured irrigation, infestation by rodents and diseases, poor postharvest management, improper marketing facilities, increased urbanization on saffron land, rampant adulteration, and clandestine smuggling of cheap saffron.<sup>2</sup> The latest and most formidable challenge that threatens the existence of saffron industry is the adverse effect of climate change.

### Environmental Stresses Caused by Climate Change

Corm sprouting, flower initiation, and time of flowering are the critical stages that are influenced by environmental fluctuations in terms of temperature and availability of water. Day temperature of 23–25 °C in the month of September is critical for corm sprouting, whereas flowering is initiated when the day temperature reaches to 17 °C with a night temperature around 10 °C.<sup>16</sup>

Saffron fields in Kashmir are entirely rain-fed. If rains fail, the crop also fails. Although water requirement is low for saffron, water stress affects yield, growth, and development.<sup>3,17</sup> The areas receiving 100–150 cm of well distributed rainfall with snow in winter are suitable for saffron cultivation, and rains in September are essential for meeting the water requirement of corms for good flower yields.<sup>18</sup> The total rainfall during the saffron growing period is usually sufficient, but its distribution is irregular, and it has now become common for saffron to face water stress. Rainfall of 100–150 mm is considered essential during the pre-flowering stage.<sup>19</sup> The flowering is optimum and saffron yields are good only when rains are received at sprouting and pre-flowering stages. Assured irrigation, at pre-sprouting and pre-flowering stages causes a quick activation of buds leading to corm sprouting

and flower initiation. Any un-seasonal rains or drop in ambient temperature during October causes serious damage to flowering, leading to heavy reduction in saffron yield (Fig. 2). Flowering occurs between mid-October to mid-November every year, mostly in three flushes in a normal season.

Due to climate change in the last some years the weather has become quite erratic and rains are either scanty or distribution is irregular, thus adversely affecting the critical stage of flowering in saffron. Kashmir faced an acute drought in 1999–2003,<sup>20</sup> and during this period productivity reduced from 3.12 kg ha<sup>-1</sup> to 1.57 kg ha<sup>-1</sup>. During 2005, favorable rainfall improved saffron productivity to 2.96 kg ha<sup>-1</sup>. Saffron is still grown as rainfed crop, and hence vulnerable to scarcity / surplus of water. The surplus of water is as detrimental as the scarcity. Heavy rains over a short period on poorly drained saffron soils pre-dispose saffron corms to rotting fungi like *Rhizoctonia crocorum*, *Phoma crocophila*,<sup>21</sup> *Macrophomina phaseolina*,<sup>22</sup> *Fusarium moniliforme* var *intermedium*, a non-sporulating basidiomycetous fungus,<sup>23</sup> *Fusarium oxysporum*, *F. solani*, *F. equiseti*, *F. pallidoroseum*, *Penicillium* sp., *Mucor* sp.,<sup>24,25</sup> and *Sclerotium rolfsii*.<sup>26</sup>

Corm rot of saffron caused by *F. oxysporum* and *F. solani* is considered to be most destructive in Kashmir.<sup>24,25</sup> These infections generally take place through the injury of corms. Infected corms possess dark-brown sunken and irregular patches below the corm scales, mostly near root and bud regions. In severe cases the entire corm turns into a black powdery mass. The foliage of infected corms shows symptoms of “die-back.”<sup>27</sup> The disease is quite widespread and causes loss of a considerable proportion of the produce every year. For controlling saffron corm rot, the corms to be planted are put in a fungicidal solution containing Carbendazim 50WP (0.1%), Mancozeb 75WP (0.3%) for 5–10 min, and then dried in shade for 10–15 min.<sup>27</sup> Similarly, Bavistin and Tecto (0.2% each) dip or drench help in disease control.<sup>28</sup> Corm treatment with Carbendazim 50WP (0.2%) or Myclobutanil (10WP) (0.2%) is also effective in reducing the corm rot severity.<sup>25</sup> These treatments are effective only for 1st year of planting in a planting cycle of 5–10 y. Even if mother corms are planted after fungicidal treatment, the daughter corms that develop in the later years get easily infected because they cannot be dug out each year to give chemical treatment. Under climate change scenario heavy rainfall over short period along with high temperature have a potential to cause heavy losses to the saffron plantation and yield, because such conditions are conducive for the growth of fungal pathogens causing rot.

#### Weed infestation

The major weeds found in saffron fields of Kashmir include *Euphorbia helioscopia*, *Lepidium virginicum*, *Salvia moorcroftiana*, *Papaver rhoae*, *Chonspora tanella*, *Galium tricornis*, *Tulipa stellata*, *Erodium cicutarium*, *Lithospermum arvense*, *Ranunculus arvensis*, *Medicago lupulina*, *Poa bulbosa*, *Crepis saneta*, *Filago arvense*, *Polygonum aviculare*, *Chenopodium album*, *Descurainia sophia*, among others.<sup>29</sup> Weed management would be an important concern in future as climate change may promote the growth of some weeds due to conducive growth conditions for vigorous growth, causing heavy losses to saffron production. Currently, no weed management practices are followed by saffron growers

except for harvesting of some weeds as fodder by farm women in May, and cattle grazing by some farmers in August.

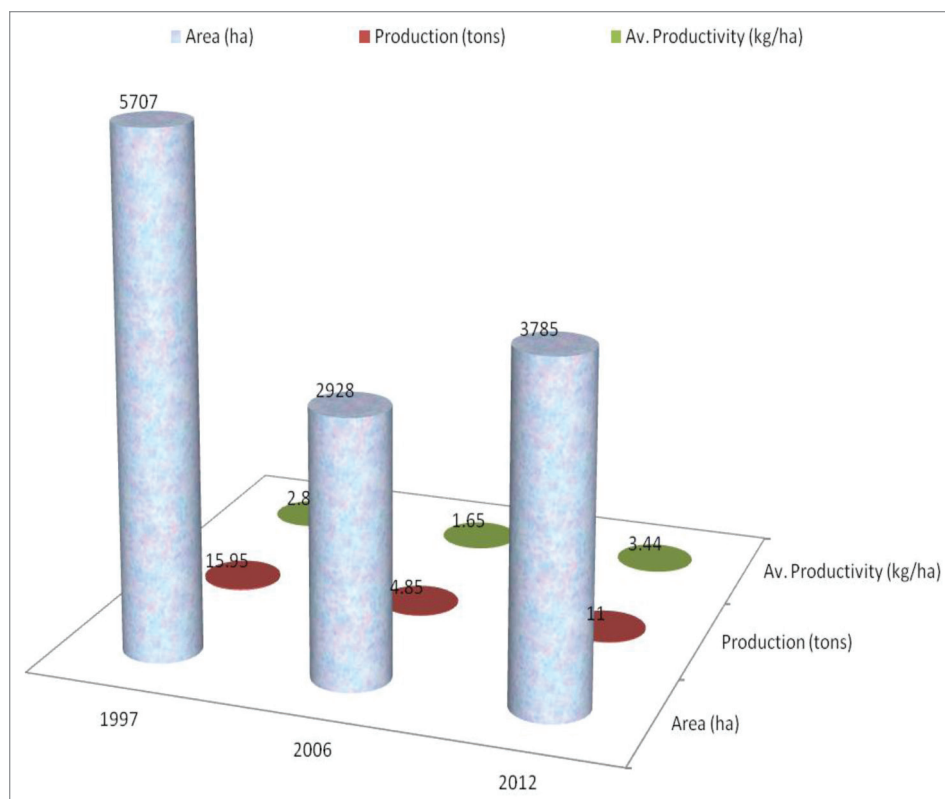
#### Saffron quality

Saffron is economically an important crop as far as its use as spice and medicine is concerned. All these biotic and abiotic stresses not only affect the productivity adversely but may also have negative impact on saffron quality (crocin, picrocrocin, and other bioactive compounds). As such, controlled climate change studies need to be conducted in SPAR (Soil Plant Atmosphere Research) facilities to find the impact of temperature, moisture and CO<sub>2</sub> on saffron quality.

### Omics Approaches for Understanding Effect of Climate Change Stresses on Saffron Biology

Saffron is a triploid species with  $3n = 24$ ,  $x = 8$  chromosomes. Its triploid nature allows for vegetative multiplication, but not regular sexual reproduction. This is because meiosis and gamete development in triploids are irregular, resulting in many anomalies in sporogenesis and gametophyte development. Saffron stigmas and corms are characterized by the presence of antifungal saponins<sup>30</sup> and defense proteins capable of binding to chitin and chitin oligosaccharides. A new class of defense chitinase namely Safchi A has been isolated from saffron.<sup>31,32</sup> Different phenolic compounds (pyrogalllic acid, kaempferol, *p*-coumaric acid, and gallic acid) involved in stress responses have also been identified.<sup>33,34</sup> Peroxidase, catalase, and superoxide dismutase activities have been detected in saffron corms in different developmental stages,<sup>35,36</sup> and genomic approaches have enabled the identification of partial sequence homologs for these enzymes. Bioinformatics analysis of corm and stigma libraries from *Crocus* has identified several genes associated with defense responses.<sup>37</sup>

Although a lot of literature can be found on botanical aspects of saffron, not much information is available on ecophysiological aspect. Several environmental parameters affect flower induction in saffron among which temperature seems to play a pivotal role.<sup>38,39</sup> Flower induction requires an incubation of the corms at high temperature (23–27 °C), followed by a period of exposure at moderately low temperature (17 °C) for flower emergence. There are evidences which show the critical importance of light and temperature in biological activities of plants including regulatory effects on dormancy period, vegetative and generative growth particularly flowering habit.<sup>40,41</sup> Transcriptome analysis



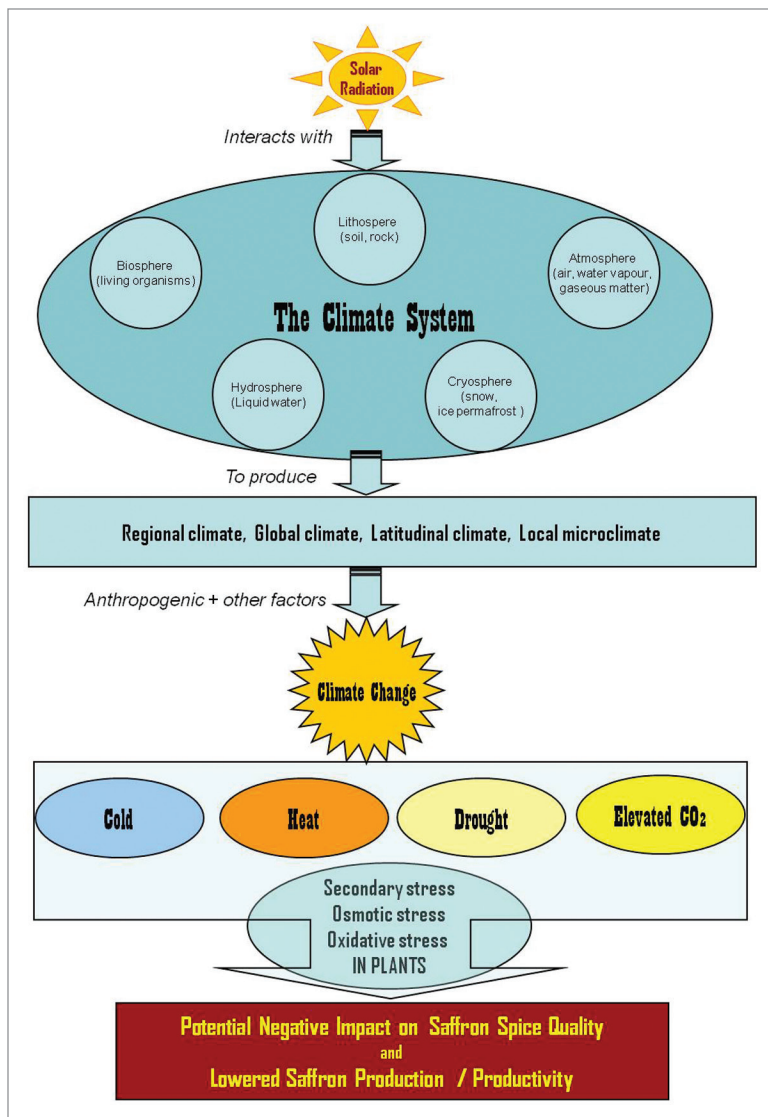
**Figure 1.** The figure shows a sharp decline in saffron area, production and productivity between 1997 and 2006 (decade), and revival since 2010 on account of National Saffron Mission.

of saffron plants subjected to different photoperiod and temperature regimes can throw light on the genes that get up or downregulated and might lead to the identification of novel regulators which influence or lead to saffron flower initiation in a particular agroclimatic condition.<sup>42</sup> How these differences and similarities translate into gene expression can be known using DNA microarray technology and bioinformatics tools. The huge database generated by physiological, agronomic, transcriptomics, proteomics, metabolomics based studies can then be analyzed by computational biology approach to find target genes for improving resilience of saffron to climate change.<sup>43</sup> This knowledge may also be used to specifically tailor saffron plants for new geographical areas with adaptability to specific climatic conditions. The problems due to the challenges of climate change to saffron production can be mitigated by use of 'alternative' sustainable technologies coupled with modern biotechnological approaches. Adoption of such technologies can be promoted by strong policy decisions.<sup>44</sup>

#### Microbial Associations for Enhancing Resilience

Changes in rainfall patterns affect soil surface temperatures and moisture availability. This can influence crop establishment, crop quality, and the total saffron production. To cushion crop production against these adversaries, symbiotic fungi can come to our rescue (Fig. 3).





**Figure 2.** Components of 'Climate' interact to create optimal plant growth conditions for Saffron; while their disturbance upsets growth and development, and lowers productivity.

During the last two decades, the mycorrhizal technology has been used to improve growth of a number of micropropagated horticultural crops as well as to enable host plants to tolerate or withstand the impairing effects of abiotic and biotic stresses.<sup>45-49</sup> Among these soil microorganisms, the most abundant and effective are rhizobia, plant growth promoting rhizobacteria (PGPR), and arbuscular mycorrhizal fungi (AMF).<sup>50</sup>

#### *Rhizobium* sp

The beneficial effects of rhizobial symbiosis on plant growth and yield under water stress conditions are bacterial-genotype specific. Swaine et al.<sup>51</sup> reported that a strain of *Bradyrhizobium elkanii* isolated from a drought environment was more tolerant to in vitro osmotic stress than strains isolated from wet environments. Mnasri et al.<sup>52</sup> found that *Phaseolus vulgaris* plants inoculated with a salt-tolerant nitrogen-fixing bacterial strain (*Ensifer meliloti*) were more tolerant to drought than those inoculated

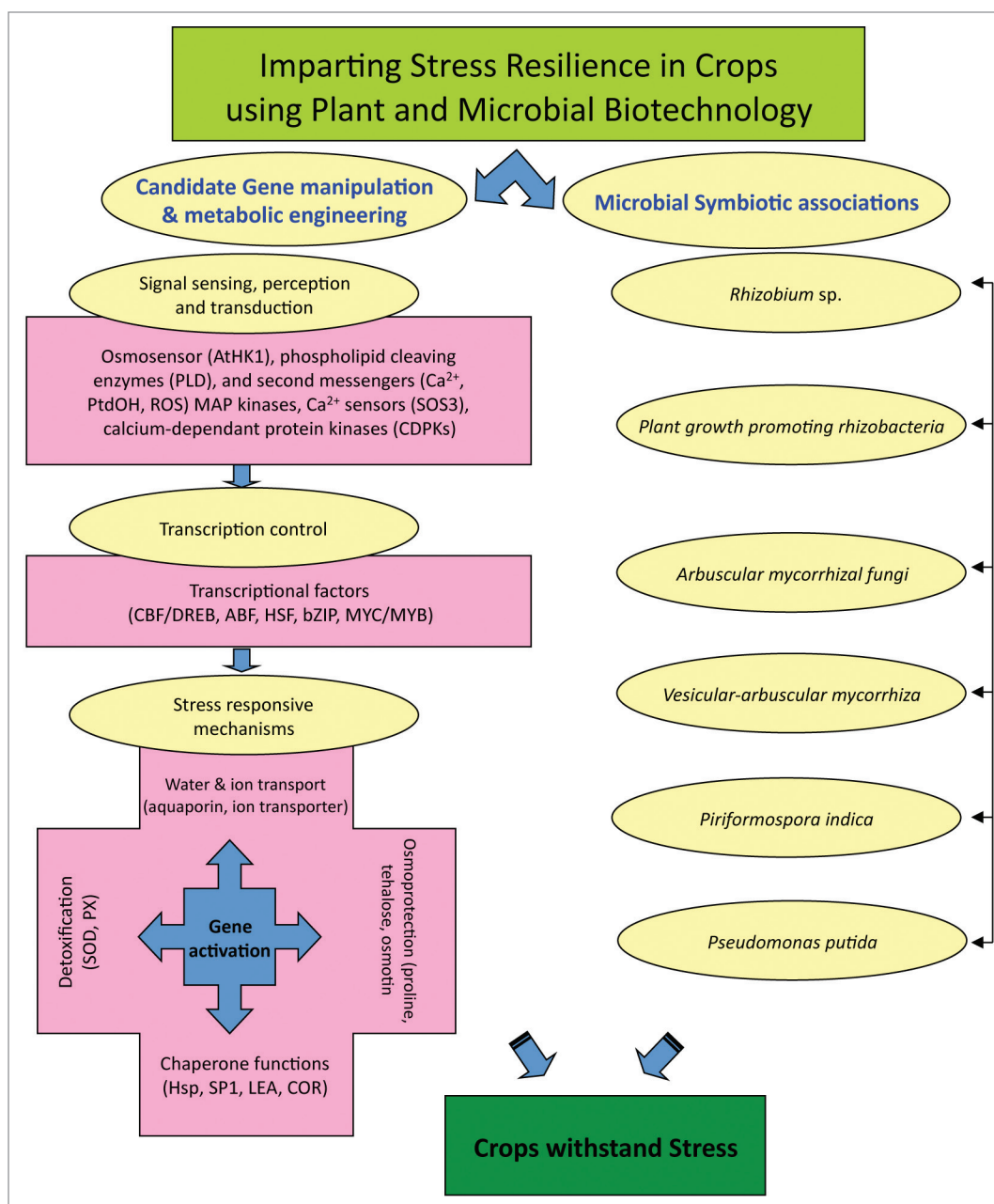
with a salt-sensitive bacterial strain (*Rhizobium tropici*). Rhizobial symbiosis has been associated with regulation of root hydraulic properties.<sup>53</sup> A diminution in the expression of an aquaporin gene in the roots of soybean plants inoculated with the *Bradyrhizobium japonicum* under both control and water-deprived conditions was observed. At the same time, it is known that some aquaporins may transport ammonia or ammonium, and hence they could be involved in the transport of nitrogen compounds between the nodules and the host plant.<sup>54,55</sup>

#### Plant growth promoting rhizobacteria (PGPR)

Plant growth promoting rhizobacteria (PGPR) represent about 2–5% of total rhizobacterial community. These are an indispensable part of rhizosphere biota which can stimulate the growth of the host.<sup>56</sup> PGPRs are candidates for establishing in soil ecosystem as these are highly adaptability in a wide variety of environments, growth rate is faster and have capacity to metabolize a wide range of natural and xenobiotic compounds.<sup>57</sup> PGPR colonize roots of plant and promote plant growth and development through a variety of mechanisms such as production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization, and promotion of the mineral nutrient uptake.<sup>58,59</sup> IAA (indoleacetic acid) is a product of L-tryptophan metabolism in bacteria and is prevalent in the rhizosphere because of the rich supplies of substrates exuded from the roots.<sup>60,61</sup> Phosphate solubilizing bacteria (PSBs) inhabiting the rhizosphere are promising biofertilizers as these are known for secretion of organic acids and phosphates to solubilise insoluble phosphate to soluble forms.<sup>62</sup> PGPR's also secrete Siderophores, in response to iron deficiency, which help in the transportation of ferric iron into plant cells from insoluble forms. They also improve antagonism against phytopathogens<sup>63</sup> and in induction of systemic resistance in plants.<sup>64</sup>

Sharaf-Eldin et al.<sup>65</sup> studied the effect of *Bacillus subtilis* FZB24 on saffron corms under ex vitro conditions in Egypt and reported that inoculation of *B. subtilis* FZB24 significantly increased leaf length, flowers per corm, weight of the first flower stigma, total stigma biomass, and significantly decreased the time required for corms to sprout and the number of shoots. In India (Kashmir) Parray et al.<sup>66</sup> isolated the associated rhizobacteria from the roots of the flowering corms of saffron on Laureia Bertani (LB) agar medium. A total of 06 isolates of rhizobacteria were selected and were identified. The rhizobacterial strains were identified as *Acinetobacter lwofii*, *Acinetobacter hemolyticus*, *Bacillus subtilis*, *Pseudomonas* ssp, *Pantoea* ssp., and *Klebsella* ssp. The *B. subtilis*, *Pantoea* ssp. and *A. lwofii* were categorized as high IAA producers. The *B. subtilis* was best strain for IAA production and shows efficient prospect for enhancing the growth and development of corms. *Pseudomonas* ssp. was found to be highly efficient in terms of phosphate solubilisation production followed by *B. subtilis*. The study also

focused on the screening of rhizobacterial strains for their potential to produce siderophore compounds. The highest percentage for siderophore production was for *Pseudomonas* ssp. (62%) followed by *B. subtilis* (59%), *Pantoea* ssp. (52%), *A. lwofii* (45%), and *Klebsella* ssp. (31%); however, *A. hemolyticus* did not show any activity. In another study, Ambardar and Vakhlu<sup>67</sup> reported that saffron rhizosphere is dominated by  $\gamma$ -proteobacteria characterized by *Pseudomonas* and *Acinetobacteria* genera. Saffron rhizobacteria and bulk soil bacteria were categorized into 16 different bacterial species with *Bacillus aryabhatai* (WRF5-rhizosphere; WBF3, WBF4A, and WBF4B-bulk soil) common to both rhizosphere as well as bulk soil. *Pseudomonas* sp. in rhizosphere and *Bacillus* and *Brevibacterium* sp. in the bulk soil were the predominant genera respectively. Three species of *Pseudomonas* isolated from the Saffron rhizosphere are specific to saffron rhizosphere, as *P. koreensis* WRF6 and *P. kilonensis* WRF3 have not been reported from any other rhizosphere and *P. tremae* have been isolated only from coffee seedlings. The isolated rhizobacteria were screened for plant growth promotion activity like siderophore, indole acetic acid production, and phosphate solubilization. 50% produced siderophore and 33% were able to solubilize phosphate whereas all the rhizobacterial isolates produced indole acetic acid. From the results, it can be concluded that the bacterial consortia have some important plant growth promoting traits that can be used as biofertilizers and application of these rhizobacterial strains may provide some benefit to saffron growers by speeding corm growth (earlier shoot emergence) and increasing stigma biomass.



**Figure 3.** Biotechnological approaches for building resilience and mitigating the negative effect of stresses imposed by climate change.

### Arbuscular mycorrhizal fungi (AMF)

About 90% of vascular plants establish symbiosis with arbuscular mycorrhizal fungi (AMF).<sup>68</sup> The association between an AMF and a plant makes the host plant more tolerant to drought in terms of plant growth.<sup>69-71</sup> These beneficial effects have been attributed to the expression of some genes related to drought stress like late embryogenesis abundant proteins (LEA),  $\Delta^1$ -pyrroline 5 carboxylate synthase (P5CS). Porcel et al.<sup>72,73</sup> found that both genes increased their expression under drought conditions more in non-AM plants than in AM ones. Similarly some superoxide dismutase isoforms from lettuce are specifically upregulated by

drought conditions in AMF-colonized plants.<sup>74</sup> Generally AMF-colonized plants have higher activities of several antioxidant enzymes than non-colonized ones,<sup>75</sup> but it depends on the enzyme activity, plant organ, and the AMF genotype involved.<sup>76,77</sup>

#### Vesicular-Arbuscular Mycorrhiza (VAM)

Many studies have shown that drought resistance of crops could be improved by vesicular-arbuscular mycorrhiza (VAM).<sup>78-83</sup> VAM fungi inoculation could slow down the reduction of chlorophyll and increase the drought resistance of plants by promoting the defense response of protective enzyme system in host plants.<sup>49</sup> Some researches showed that the formation of VAM could enhance the activity of antioxidant enzymes of SOD, POD, CAT, and H<sup>+</sup>-ATPase in host plants and reduce the content of MDA and electrical conductivity of plasma membrane significantly,<sup>84,85</sup> but it is still not clear about the mechanism of VAM to enhance the drought resistance of plants.

Since most arbuscular vesicular-mycorrhizal fungi are obligate biotrophes in nature, they are difficult to culture in vitro, and this is a major obstacle to their practical use.<sup>86</sup> A traditional method of producing large amounts of inoculum is to culture the fungi on roots in soil,<sup>87</sup> but this method is very tedious and time-consuming.

#### *Piriformospora indica*

*Piriformospora indica* (Hymenozymetes, Basidiomycota) is a wide-host root-colonizing endophytic fungus which allows the plants to grow under extreme physical and nutrient stress. This novel multifunctional symbiotrophic fungal endophyte was discovered from the Great Indian Desert of Western Rajasthan in India.<sup>88</sup> The fungus was found to colonize roots of the desert plants which were growing in extreme conditions of water scarcity. Subsequently, it was established that the fungus promotes acquisition of drought tolerance in plants.<sup>89,90</sup> In contrast with arbuscular mycorrhizal fungi, it can be easily cultivated in axenic culture where it produces chlamydozoospores.<sup>91-93</sup> It is easily cultivated on a number of synthetic and complex media.<sup>94,95</sup> Being the only cultivable endophyte that colonizes roots, this fungus provides a model organism for the study of beneficial plant-microbe interactions and a new tool for improving plant production systems.<sup>88,96,97</sup>

*P. indica* has a vast geographical distribution and is reported from Asia, South America and Australia. The fungus has a huge potential for biotechnological applications, especially under changing climate scenario because it functions as a biofertilizer in nutrient-deficient soils; as a bioprotector against biotic and abiotic stresses; as a bioregulator for plant growth development and enhanced seed production; and as a bio-agent for the hardening of tissue-culture-raised plants.<sup>98</sup>

#### *Pseudomonas* spp

*Pseudomonas putida* and *P. fluorescens*, the common PGPR of most of the plants, are absent in Saffron rhizosphere though *P. koreensis* WRF6, which shows maximum production of the indole acetic acid (28.5 lg/ml) is comparable to the other common growth promoting Pseudomonads e.g., *P. putida* (24.08 mg/l) and *P. fluorescens* (31.6 mg/l).<sup>67</sup> The inoculation of saffron corms with *P. koreensis* WRF6 may provide protection against the adverse effect of temperature rise on saffron plants, in a

manner similar to that observed in sorghum and pearl millet. It has been found that seed inoculation with stress-tolerant strain of *Pseudomonas putida* helped sorghum and pearl millet seedlings survive at 50 °C up to 21 d, whereas the controlled seedlings could survive only up to 10 d (DARE-ICAR Report 2008–09; <http://www.icar.org.in>). This remarkable protective effect was mediated through induction of the synthesis of novel high molecular weight proteins in the leaves, which were not found in the controlled seedlings. Inoculation also reduced the oxidative stress in seedlings exposed to high temperature (50 °C) as evidenced by significantly lower oxidative enzyme activity in treated seedlings. The introduced organism successfully entered into the roots and induced the physiological changes at the whole plant level as confirmed by electron micrography (DARE-ICAR Report 2008–09).

### Genetic Modification and Genome Engineering

Five primary environmental factors are critical for plants to germinate, grow, and reproduce, and saffron is no exception. These factors are carbon dioxide, sunlight, water, optimal temperature range, and nutrients. Of these, the first four are directly related to climate and vary spatially, diurnally, and seasonally. In the context of global climate change these factors behave erratically and, therefore, need to be considered foremost while designing saffron plants adapted to changing climatic conditions. Tolerance to heat, drought, water-logging, salinity, and frost; resistance to pests and diseases; water-use and nitrogen-use efficiency are the most important traits for adaptation to climate change.<sup>1</sup> Genetic modification techniques are becoming increasingly feasible due to improved techniques and demystification of the various side effects of transgenic technology.<sup>99,100</sup> An array of genes, available for use in both cisgenic and transgenic approaches, can be used for the development of varieties with better resilience to vagaries of climate (Fig. 3). Clustered regulatory interspaced short palindromic repeats (CRISPRs) and CRISPR-associated (Cas) systems is another emerging technique that can be employed to introduce useful genome modifications through genome engineering of saffron. These are revolutionizing the field of genome editing.<sup>101</sup> With their highly flexible but specific targeting, CRISPR-Cas systems can be manipulated and redirected to become powerful tools for genome editing.

### Conclusion

Using biotechnology, agronomic practices that increase carbon sequestration can become easy to adopt and as such render additional benefits too, like increased root biomass, soil organic matter, water and nutrient retention capacity, and, hence, increased saffron productivity. Enhancing carbon sequestration capacity in degraded saffron lands through organic means could thus have direct environmental, economic and social benefits for local people, with consequent improvement in their livelihood and food security status.



Establishment of symbiotic relationships between microorganisms and plants has shown promising potential for enhancing tolerance of plants to disease and drought. The beneficial effect of mycorrhization on plant drought tolerance is caused by the development of superior root system, enhanced water conducting capacity, increased uptake of macro, micro, and immobile nutrients, resulting in better carbon dioxide assimilation and higher photosynthetic rates. Among the promising bacteria that can be included into the saffron management practices *Pseudomonas* ssp. and *Bacillus* ssp. are particularly important owing to their beneficial influence. Among the symbiotic fungi, *Piriformospora indica* and *Sebacina vermifera* should be studied as these have been shown to mimic the capabilities of AMF and are axenically cultivable on a number of synthetic and complex media.

Data generated by physiological, agronomic, transcriptomics, proteomics, metabolomics studies can help find target candidate genes for improving resilience of saffron to climate change. Modern Genetic modification techniques including CRISPR can

be used to create saffron plants that possess inbuilt tolerance to biotic and abiotic stresses, and hence make these plants resilient to some adverse effects of climate change.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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