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Climate resilience initiatives at Indian Institute of Vegetable Research

A large shift in global climate will have an influence on vegetable cultivation and agriculture as a whole, affecting the world's food supply. Climate change will have an impact on the intensity of environmental stress imposed on vegetable crops, and rising temperatures, decreased irrigation water supply, and floods will be important limiting factors in maintaining and expanding vegetable production. Development of varieties, technologies and intervention of biotechnology approaches are the options to meet the challenges due to changing climatic scenario.

LAST three decades, vegetable output has risen globally, surpassing grain production in terms of value of global commerce. The main factor behind agricultural losses globally is environmental stress, which reduces major crop yields by more than 50%. The environment that vegetables are produced in varies tremendously depending on the season and the location. Plants may use morphological or biochemical strategies to react similarly to avoid one or more stresses. For the production of tropical vegetables to be sustainable, adaptation measures are essential. The current cultivation of vegetables faces major obstacles and harmful effects from climate change. These obstacles and issues with vegetable crops are given below.

- **Temperature:** High temperatures are associated with bud drop, abnormal flower development, poor pollen production, dehiscence, viability, ovule abortion and reduced viability, reduced carbohydrate availability, other reproductive abnormalities, small and poor quality. Reduced fruit set, can further significantly reduce vegetable productivity.
- **Excess moisture:** In general, floods harm vegetable output. The depletion of oxygen in the root zone, which limits aerobic activities, is a major source of productivity loss. The intensity of flooding symptoms worsens as temperatures rise; fast withering and death of plants is common after a brief period of flooding at high temperatures.
- **Moisture deficient:** Water resources are expected to be very sensitive to climate change and severe water shortages will affect crop yields (affecting yield and quality), especially for vegetables. Most vegetables are more than 90% water and for this reason water scarcity leads to large yield losses in vegetables.
- **Salinity:** Salinity is a serious issue that has an impact on crop productivity all over the world. Climate change, excessive groundwater consumption (especially near the sea), growing irrigation with low-quality water, and widespread implementation of irrigation linked to intensive farming can all exacerbate this process. Sensitive vegetables suffer from excessive soil salt, which lowers their production.

Keeping in view the above facts, initiatives have been taken for Climate resilience in vegetable crops at ICAR-Indian Institute of Vegetable Research as described below.

(A) Climate resilient varieties developed

Tomato

Kashi Adabhut: This hybrid was developed and recommended by State Variety Release Committee in 2022 for commercial cultivation in Uttar Pradesh. Plants are determinate in growth. Fruit setting occurs at high temperature >34/18°C (day/night) and maturity starts 100-105 days after transplanting. Transplanting is done in first week of February and fruits harvesting continues up to third week of June. Fruit size (45-60 g) and fruit colour (red) are maintained up to last picking. Average yield is 37.2 tonnes/ha which is 54.5% more than local check Aryan-60. The physiological losses were 9.11% during

storage for six days after harvesting, at breaker stage (in room day temperature of 35±3°C).

Kashi Tapas: This hybrid was also developed and recommended by State Variety Release Committee in 2022 for commercial cultivation in Uttar Pradesh. Plants are in-determinate in growth. Fruit setting occurs at high >34/18°C (day/night) temperature and maturity starts 100-105 days after transplanting. Transplanting is done in first week of February and fruits harvesting continues up to first week of June. Fruit size (40-45 g) and fruit colour (red) are maintained up to last picking. An average yield is 44.49 tonnes/ha. The physiological losses were 10.69% during storage for six days after harvesting at breaker stage (at room day temperature of 35±3°C).

Cowpea

Kashi Nidhi (VRCP-6): This variety was developed through hybridization followed by pedigree selection of cross Kashi Unnati × Cowpea-263 and released by Central Variety Release Committee in 2012 for commercial cultivation. This variety is dwarf photo-insensitive and tolerant to low temperature up to day temperature of 15°C and continues flowering and fruiting up to second week of December. It is also tolerant to cowpea golden mosaic virus and *Cercospora* leaf spot under field conditions. An average green pods yield is 125-150 q/ha.

French bean

Kashi Sampann: This variety was released and notified by State Variety Release Committee in 2019. It has ability to set fruits at 32-34°C, hence off-season green pods availability is up to second week of March. This is true vegetable type which yields 24.9 t/ha (green pods). Also, it is tolerant to French bean golden yellow mosaic virus under field condition.

Kashi Rajhans: This variety was released and notified by State Variety Release Committee in 2019. This is a true vegetable type which yields 24.13 t/ha (green pods). It has ability to set fruits at 32-34°C, hence off-season green pods availability up to second week of April, when other varieties of pods are not available. It is tolerant to French bean golden yellow mosaic virus under field conditions.

Indian bean

Kashi Bouni Sem-3: Developed through inter-varietal of Kashi Haritima (Pole) × Arka Vijay (Bush) followed by pedigree and individual plant selection and has ability to give flo-wering–fruiting at day temperature of up to 35°C. Determinate (Bush type) in growth (plant height 80-85 cm). Seed sowing period is last week of September to last week of October (*Rabi* crop). Peak fruiting period is in December last week to March first week. Days to first picking is 80-85. Staking is not required. National average production is about 257.48 q/ha in crop duration of 140-150 days. Free from Dolichos yellow vein mosaic virus during cropping period.

Kashi Bouni Sem-9: This variety has also been developed through inter-varietal Kashi Sheetal (Pole) × Gomachi Green (Bush) followed by pedigree and individual plant selection and has ability to give flowering–fruiting at day temperature of up to 35°C. Growth habit is determinate (Bush type) with a plant height of 70-75 cm. Seed sowing period is last week of September to last week of October (*Rabi* crop). Peak fruiting period is last week of November to last week of December and continues to first week of March. Staking is not required. National average production is about 241.01 q/ha in crop duration of 140-150 days. Free from Dolichos yellow vein mosaic virus during cropping period.

Radish

Kashi Rituraj: ICAR-IIVR, Varanasi, has developed a CMS-based F1 hybrid of radish, first among Public Sector in India, which has ability to tolerate high temperature up to 38-43°C during April-June (summer) in North Indian plains; and has wider adaptability, i.e. suitable for cultivation during winter, spring, summer and autumn seasons. It produces lust-green leaves with sinuate type of leaf morphology which is preferred by consumer; forms attractive-white and long roots; and has higher marketable yield potential, i.e. 600-700 q/ha during winter season, 450-550 q/ha during spring season, and 350-425 q/ha during summer and autumn seasons.

Kashi Rituraj: Field view during summer season (May-June) at Varanasi

Kashi Rituraj has been released for Uttar Pradesh by SVRC in October 2022, and its female parent is a CMS line (VRRAD-201) which has been registered as Unique Germplasm (INGR20032) by ICAR-NBPGR, New Delhi in 2020.

Water spinach

Kashi Mannu: This variety can be grown throughout the year and can withstand extremely high temperature, drought, cold and water-logged conditions along with low input cost required for raising the crop. First variety for upland field conditions and fast growing. First harvesting starts 20-25 days after seed sowing. Leaf biomass yield of 693.0-1000 q/ha; multiple cuttings (3-4 cuttings/ month).

(B) Intervention through grafting technology and use of plant growth substances

Grafting tomato on brinjal rootstocks for alleviating water-logging stress: Vegetables like tomato, brinjal, watermelon, melon and cucumber can readily be grafted onto different inter-specific rootstocks with objective to reduce infections by soil-borne pathogens and to enhance the tolerance to abiotic stresses, such as soil salinity, drought, thermal and water-logging. For early tomato production in northern Indian plains, it is transplanted during south-west monsoon period (August-September) when persistent heavy rainfall with poor drainage induces water-logging situation. Water-logging coupled with high ambient temperature increases the temperature of stagnated water, resulting in quick plant wilting. Tomato is considered one of the most susceptible vegetable crops to excessive soil moisture/ water-logging and excess water which can produce hypoxic and anoxic soil conditions within few hours. To overcome water-logging situation and thereby early production of tomato, grafting study was conducted at ICAR-IIVR, Varanasi during 2013-18. Several rootstocks of brinjal (cultivated and wilds) were evaluated to water-logging tolerance in tomato. Both grafted and non-grafted plants were exposed to water-logging stress for 48 to 120 hour both during vegetative and reproductive stages. All brinjal genotypes showed some tolerance to water-logging situation, but two rootstocks, i.e. IC-111056 and IC-354557 were identified as most promising because tomato grafted on these rootstocks did not show any wilting or yellowing, and also maintained higher CCI, chlorophyll fluorescence yield with optimum enzymatic and non-enzymatic biochemical traits when exposed for 72 hour during vegetative stage (August- September; 30-40 DAT) and 96-120 hour during reproductive stage (November-December; 45-60 DAT). In contrast, the non-grafted tomato plants registered 39.6-41% reduction in Fv/Fm, and 41-100% reduction in CCI at 4 days after relieving from water-logging stress. Non-grafted plants completely wilted and died in 4-5 days after relieving from water stress; whereas the grafted plants recovered completely from 96 hour of water-logging stress shock, 3 days after relieving from the stress. This technology has also been demonstrated successfully at 24 farmer's fields in 5 villages of Mirzapur and Varanasi districts of Uttar Pradesh.

Use of plant growth substances (PGS) for high temperature tolerance: In tomato, fruit set is badly hampered by the high Day/ Night (D/N) temperatures. It has been reported that fruit set in tomato is reduced drastically if D/N temperature is 34/18°C. ICAR-IIVR has developed two tomato hybrids, i.e. Kashi Adbhut (VRNTH 18283) and Kashi Tapas (VRNTH 19085) that can set fruit at high temperatures (>38/26°C D/N). Plant growth substances (PGS) are also known to enhance fruit set in adverse climatic conditions. At ICAR-IIVR, Varanasi, an experiment on use of PGS in tomato was conducted during summer season (February to June) in 2021 and 2022 when day temperatures reach more than 42°C with night temperature of 30-32°C. In this study, ICAR-IIVR evolved 11 hybrids/advance lines of tomatoes which were evaluated with 3 private-sector hybrids under high temperature condition. Tomato plants were sprayed with 4 different PGS such as proline (10 µmol/L), salicylic

(a) Grafted plants exposed to waterlogging situation and (b) grafted plant distribution to the farmers

High temperature tolerant hybrid tomatoes developed from ICAR-IIVR, Varanasi

acid (SA 250 µmol/L), sodium-nitro-prusside (SNP 25 µmol/L) and gibberellic acid (GA 50 ppm). PGS was sprayed thrice, i.e. 30, 45 and 60 days after transplanting. Experimental findings revealed that maximum fruit set (67.35%) was recorded in Kashi Adbhut variety with use of SNP, whereas the maximum number of fruits/plant was noticed in Kashi Adbhut with use of SA (72.6) followed by SNP (62.9). As far as fruit yield is concerned, the maximum fruit yield of 18.3 kg/plant and 1.81 kg/plant were reported, respectively in Kashi Adbhut (VRNTH 18283) and VRNTH 20132 with spray of SNP.

Use of plant growth substances (PGS) for moisture deficit condition: Five moisture deficit tolerant F1s, viz. VRNTH-18-1, VRNTH-18-2, VRNTH-18-3, VRNTH-18-4 and VRNTH-18-5 along with three private-sector hybrids were exposed to moisture deficit conditions at vegetative and reproductive stages. The plants were sprayed with 3 different PGS, i.e. salicylic acid (SA-250 µM), sodium nitro-prusside (SNP-25 µM) and spray of both SNP and SA under irrigated moisture deficit condition (withholding irrigation for 21 days both at vegetative and reproductive stages). Data revealed that 5 hybrids, viz. VRNTH-18-1, VRNTH-18-2, VRNTH-18-3, VRNTH-18-4 and VRNTH-18-5 were superior over popular private sector hybrids in terms of yielding ability in moisture deficit conditions. The identified hybrids yielded in a range of 53–59.33 t/ha. Tomato hybrids VRNTH-18-1 and VRNTH-18-2 with combined spray of SA+SNP yielded 30-47% higher than the most popular private sector hybrids being grown locally.

(C) Biotechnology intervention for development of climate resilience vegetable crops

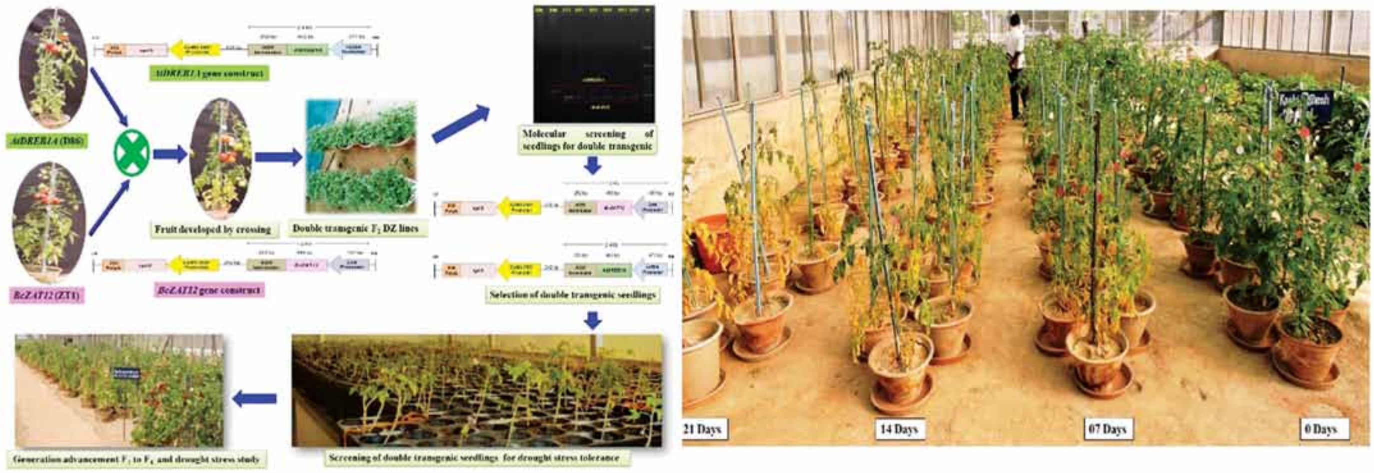
Water use efficiency of AtDREB1A and BcZAT12 transgenic tomato lines: Drought tolerance has a major impact on more sustainable cropping systems worldwide, mainly in developing countries like India, where drought will likely be more prevalent and severe. Drought transgenic tomato lines containing AtDREB1A or BcZAT12 gene were developed. To compare water use efficiency (WUE) of these transgenic lines with drought tolerant tomato genotypes, AtDREB1A events (D41, D53, D76, D86, and D90), BcZAT12 events (ZAT1, ZT2, ZT5, and ZT6), non-transgenic line (WT; cv. Kashi Vishesh), drought tolerant genotypes (H88-78-1 and VRT32-1), and drought susceptible genotypes (EC520046 and EC620598) seeds were germinated. The trial was conducted in an insect proof screen house. Both the transgenic lines had significantly higher fruit yield (40% FC = 36-58% higher) and more number of fruit under drought conditions. The fruit characters of transgenic lines have higher TSS, sugar, ascorbic acid, citric acid, flavanoids, and carotenoids. The *p*H of transgenic fruits were lower than non-transgenic plants. Transgenic fruits were more firm than other genotypes. DREB, DT1 and DT2 fruits of stressed plants became harder.

The pollen viability of transgenic lines showed higher viability under 40% field capacity moisture stress. The transgenic AtDREB1A events, 89.5 %; BcZAT12 events, 88.4%; D86 × ZT1 F1, 90.3%; DT genotypes, 72.3% and WT line, 70.7%.

The above results suggested that drought tolerant AtDREB1A and BcZAT12 lines were much better adopted during 40% field capacity condition compared with other drought tolerant genotypes.

Salt stress tolerant transgenic tomato seedlings–BcZAT12 gene: Abiotic stress like salinity affects tomato plant's growth and productivity in coastal and salt prone areas. Our earlier results of BcZAT12 transformed tomato lines can withstand drought stress and heat-shock stress making these BcZAT12 transgenics of tomato useful for improving quality of tomato in heat or drought stressed regions. BcZAT12 gene under the regulatory control of the stress inducible Bclea1 promoter works in multiple stress condition. Another experiment with salt stress was applied to BcZAT12 transformed as well as control tomato seedlings by adding 50, 100 and 200 mM NaCl. Morphological, physiological and biochemical characters of transgenic and non-transgenic tomato seedlings were estimated after stress application. 50, 100 and 200 mM salt stressed ZT (BcZAT12-transformed) lines (ZT1, ZT4, ZT5 and ZT6) and NT (H-86 Control) tomato seedlings were evaluated for leaf area, root and shoot length. Physiological character of electrolyte leakage, relative water content and

Water use efficiency experiment trial of AtDREB1A and BcZAT12 transgenic tomato lines within screen house



Development of drought, cold, heat and salt tolerance AtDREB1A::BcZAT12 transgenic tomato

chlorophyll colour index were estimated for stressed ZT and NT tomato seedlings. Biochemical analysis of H_2O_2, proline and catalase were observed from leaves of stressed ZT and NT tomato seedlings. Results showed reduced leaf area and shoot length with increase in root length both in NT and ZT seedlings on increasing salt stress condition whereas line ZT1 and ZT5 showed less reduction of leaf size at 200 mM stress compared with their respective NT plants whereas root length was significantly higher at all the stress conditions in line ZT1 and ZT5. Physiological parameter, viz. CCI and electrolyte leakage increased with increase in salt stress and it was higher in lines ZT1 and ZT5 both in 100 and 200 mM condition. RWC decreased with increasing salt stress condition and it was lower at 200 mM condition. ZT lines showed much more water retention capacity compared with NT plants. H_2O_2 free radical was higher in NT plant during the stress condition and it was significantly lower in lines ZT1 and ZT5 during all the stress conditions. CAT activity was significantly higher in ZT1 and ZT5 compared with their NT counterpart. Proline accumulation was significantly higher in line ZT1, ZT4 and ZT5 compared with NT plant at 100 mM salt stress condition whereas at 200 mM only line ZT1 had significant proline accumulation.

Development of double transgenic tomato expressing AtDREB1A and BcZAT12 gene for multiple stress tolerance: To develop multiple stress tolerance in tomato (cold, drought, salt and high temperature), *AtDREB1A* and *BcZAT12* genes were pyramided to obtain double transgenic tomato by reciprocal cross-pollination. The best T6 homozygous event of single transgenic plants, viz. D86 (cold, drought and salt stress tolerance) and ZT1 (drought, heat and salt tolerance) harbouring *AtDREB1A* and *BcZAT12* gene respectively were grown up in 7 kg pots containing 1:1 ratio soil and sand in transgenic glass house at 24/20°C, 16/8 photoperiod and regularly irrigated with tap water. Both the transgenics were crossed with each other in reciprocal manner. The F1 seeds were collected from ripened fruits and sown in pots for nursery raising. DNA isolated from the seedlings was screened for the *AtDREB1A* and *BcZAT12* genes presence with gene specific primers. The homozygous seedlings, harbouring both the genes were designated as *DZ-1* to *DZ-720* and screened for drought stress tolerance at seedling stage; 120 double transgenic lines were selected on the basis of visual observation (wilting) and advanced to F3 generation, and from these, five best lines were selected for drought stress experiment in F4 generation. Co-overexpressing of *AtDREB1A* and *BcZAT12* in five (DZ1-DZ5) double transgenic (DT) tomato lines was observed under 0, 7, 14 and 21 Days of Water Deficit (DWD). The DT plants showed enhanced drought tolerance and yield potential than single transgenic (ST) and non-transgenic (NT) plants. Furthermore, *AtDREB1A* and *BcZAT12* co-overexpressed plants showed reduced level of electrolyte leakage (EL), hydrogen peroxide and membrane lipid peroxidation and elevated level of relative water content (RWC), proline, chlorophyll colour index (CCI) and photosynthetic efficiency as compared to ST and NT. The plant growth and yield attributes were improved by the co-overexpression of *AtDREB1A* and *BcZAT12* in DT plants. The transcript analysis showed the increased level of *DREB1A*, *ZAT12* and *P5CS* genes expression which were higher in DT tomato plants and indicated that both the genes were induced together in the DT plants.

SUMMARY

Vegetables in general are very sensitive to extreme environmental conditions, so high temperature and limited soil moisture are the main causes of low yields and will be exacerbated by climate change. Improving the adaptability of climate-resilient growing vegetables and stress-tolerant abiotic crops, plastic tunneling technology, climate resilience through genomics and biotechnology, stress engineering and grafting to changing climatic conditions is a huge undertaking. Science and technology information developed through these initiatives should be easily accessible, consolidated, and strategically used.

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