

## Streamlining Different Plant Breeding Methods to Enhance Water Use Efficiency in Agricultural Systems

**Comment [IA1]:** Enhancing water use efficiency in agricultural practice to solve water scarcity.

### Abstract

Relevance of Water in human life and agro-ecosystems has been extensively illustrated for decades around the globe. Despite the reality that the earth is mostly surrounded by water, its utility has been limited in domestic consumption and agricultural purposes. Thus, tweaking water usage in agricultural systems has been a tricky problem in order to meet the requirements of the growing population and to obtain more crop yield per drop of water in agriculture. In this review, an attempt has been made to revisit the methods available to improve water use efficiency in agriculture.

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### Introduction

Water is essential for life [1]. The planet earth is often regarded as 'blue planet' as water occupies around 71% of its surface. But around 96% of total earth's water is saline which is unfit for domestic consumption or for agricultural purpose. Of the total fresh water content, 68% is in the form of glaciers and only 30% is in the ground. So, the amount of fresh water that sustains terrestrial life is very small, constituting less than 1% of the earth's total water content (Grey *et al.*, 2013 and Valavanidis, 2019). However, with the increasing global population, the water shortage is also increasing at an alarming rate (Jury and Vaux, 2005 and Ruggiero *et al.*, 2017).

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Water scarcity has become a major problem even for domestic use. On the other hand, the increasing population is also pressurizing the agriculture sector to increase the food production (Islam and Karim, 2019 and Shah *et al.*, 2021). But changing climate, long dry spells, and erratic rainfall pattern has become a nightmare from the recent decades and are limiting the gains in crop production. So, in order to meet the growing food demands, intensive agriculture system has come into practice which includes use of hybrids, chemical fertilizers and artificial irrigation as major inputs.

Around 80% of the world's total allocatable water resource is currently consumed by irrigated agriculture (9). So, agriculture has become the largest consumer of available water (Sharma *et al.*, 2015 and Zhu *et al.*, 2019). The total evapo-transpiration from agricultural lands is expected to increase twice in next 50 years, if we continue with the same trend of cultivation practices and food consumption (Sharma *et al.*, 2015). So, optimal management of world water resources is one of the major challenges of the current century to ensure food security and water availability to our future generations (Zhu *et al.*, 2019). In this context, there is a pressing need to improve water productivity. Producing more crop per drop of water has become the agenda in agriculture sector (7). Increasing water use efficiency of food crops offers a key to achieve this and solve the global water scarcity problem for crop production.

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### Water Use Efficiency:

As a normal physiological activity, plants diffuse carbon dioxide and water through their stomatal pores by the process of photosynthesis and transpiration respectively (14) and (10). Both these processes, being affected by the leaf area, are inter-connected with each other (10). Of the total water uptaken by the plant, over 90% is lost through transpiration and not utilized for biochemical processes (Ruggiero *et al.*, 2017). The amount of biomass produced per unit of water transpired is termed as "Transpiration efficiency (TE)" (13). Whereas, the amount of biomass produced per unit of water used by a plant is water use efficiency (WUE) (16).

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The concept of water use efficiency was first introduced by Briggs and Shantz (1913) (5) and is broadly defined as the ratio of water used by the plant for metabolism to the water lost through transpiration (Ruggiero *et al.*, 2017). But Plant metabolism is affected by rising temperatures and recurring droughts. The higher temperatures not only alter the thermal environment of plants, but make the atmosphere drier, thus increasing the evapo-transpiration and reducing WUE (11). Severe droughts also have negative impacts on growth, physiology, and reproduction of plants and lead to decline in crop yields (12).

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With the recurring events of erratic rain fall, severe drought and elevated temperatures, it is a challenging task to increase economic productivity of food crops with the limited availability of water resource. Hence, several physical and biological approaches should be pursued in an integrated manner. Besides proper agronomic practices, employing

suitable breeding methods for improving TE or WUE to boost crop yields could be a key to address this global issue of water shortage (10).

### Breeding for increased WUE or TE

Breeding for increased WUE or TE demands screening for physiological traits like root architecture, seedling vigour, days to flowering, carbon isotope discrimination (CID), leaf turgor, stomatal conductance, abscisic acid (ABA), osmolytes, chlorophyll concentration, and remobilization of water-soluble carbohydrates. Thus, there is a need to bridge the gap between physiology and breeding (Shunmugam *et al.*, 2018). There are several reports of efforts on improving WUE and drought tolerance in crop plants. With the progress made in understanding drought adaptive mechanisms of crops, the mechanism has been broadly classified into drought escape, drought avoidance, and drought tolerance based on the plant physiological habit and drought response (Sreeman *et al.*, 2018).

Drought escape is a drought adaptive mechanism which refers to the ability of the plant to complete its life-cycle before onset of drought (Shavrukova *et al.*, 2017). Early reproductive phenology is one of the major traits that help plants to escape drought (Shavrukova *et al.*, 2017 and Khan *et al.*, 2018). Early reproductive phenology here denotes for early flowering, early pod/seed development and early pod/seed maturity. For example, ILC 1799 a chickpea variety that possess early maturing habit, large seed size, higher yield, and escapes drought (Sabaghpour *et al.* 2006).

In contrast, Drought avoidance is the ability of plant tissues to maintain relatively higher water content even in water deficit conditions. This is mainly achieved by minimizing water loss (water savers) and optimizing mechanism exhibited by plants in response to drought, and is associated with small or closed stomata with reduced photosynthesis (Shavrukova *et al.*, 2017). Reduced leaf size, waxy and thick leaf cuticle layer, thick palisade tissues, large number of trichomes, and developed vascular tissues in leaves reduces transpiration and thus checks water loss under drought environment (Ilyas *et al.*, 2021). Thus, these traits form the basis of selection in breeding for arid and semi arid crops.

Drought tolerance refers to the ability of a plant to adapt itself to water deficit conditions. It includes certain biochemical or morphological adaptations of the plants and cell injury avoidance [3]. Roots are the first organ that experience the drought stress. So, the root system has a critical role in response to drought stress. Longer water uptake (4). It is the slow

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growth roots are more favorable for drought tolerance as compared to shorter roots (Ilyas *et al.*, 2021). Root traits confer grain yield advantages under terminal drought in chickpea [2]. Breeding for the best combination of profuse Root Length Density at surface soil depths, and Root Dry Weight at deeper soil layer is the best selection strategy, for high WUE and an enhanced terminal drought tolerance in case of chickpea [2]. Stay green trait in case of cereals like sorghum and pearl millet is an improved drought tolerance trait that is characterized by delayed leaf senescence and extended duration of photosynthesis (Kimani, 2012 and Serba and Yadav, 2016). The plants possessing stay green trait overcome terminal drought stress by retaining their green leaves even after anthesis for longer time and also exhibit longer grain-filling period than the non-stay green types. Thus, the plants showing stay green nature yield higher even under the harsh drought conditions (Kamal *et al.*, 2019, Serba and Yadav, 2016 and (8)

Knowing the relevance of different plant physiological traits affecting drought response *via* drought escape, drought avoidance and drought tolerance, proper breeding method has to be followed. The conventional breeding includes screening of varieties for drought resistance and identifying genetic variability to drought among crop varieties, or among sexually compatible species. Upon identifying the lines/ varieties showing drought resistance, the trait has to be introduced in the background of an agronomically superior variety. Over the decades, the conventional breeding for drought has become successful. But, it is a slow process and is limited by the availability of suitable genes in the crossable species for breeding.

The neglected and under-explored plant species serve as a reservoir of several useful genes (Padulosi *et al.*, 2013) Exploiting such species with enhanced drought tolerance, and desirable agronomic and nutritive characteristics than the cultivated varieties can be a profitable approach. Introducing such species into existing cropping systems in arid and semi-arid regions will ensure higher WUE or TE (Rosero *et al.*, 2020). But, cross-incompatibility of wild species or related species with the cultivated pool and the problem of linkage drag are the major issues hindering the progress of utilizing these gene rich species through conventional breeding approaches.

With the advancements in genome sequencing and availability of robust molecular marker systems, the breeding process has become much faster compared to the conventional breeding tools. Various works have been reported on the identification of genes and QTLs (Quantitative trait loci) controlling drought tolerance. Few to quote, 15 genomic regions

controlling traits related to drought tolerance in chickpea (Rehman *et al.*, 2017), 7 candidate genes governing stay green types in sorghum (1), and a major QTL for tolerance to water deficit condition in pearl millet (Tharanya *et al.*, 2018). The identified genes or QTLs can be transferred to the desired genotype using marker assisted breeding (Serraj *et al.*, 2005). Over expression genes/qtls/miRNA (Micro RNAs) controlling drought tolerance is yet another approach assisting in developing drought tolerant genotypes (Shah and Ullah, 2021). Over-expression of miR408 in case of chickpea (15) and miR172c of soybean (Li *et al.*, 2016) has increased drought tolerance. But, over-expression of genes may affect the other untargeted genes also, in a negative manner (Prelich *et al.*, 2012). Because of this off-target effect of over-expression technique, targeted approaches like gene editing tools have come into practice.

Of the various biotechnological tools assisting in crop breeding, gene editing technologies are gaining much popularity now-a-days. Through gene editing technologies like Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALEN), and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-Associated nuclease protein (CRISPR/Cas) system new alleles can be generated by inducing point mutations (Rosero *et al.*, 2020). The CRISPR/Cas 9 tool has been successfully employed to alter the traits related to drought tolerance in crops like soybean (6), maize (Shi *et al.*, 2017) and chickpea (3). The improvements in gene editing techniques have assured the transgene free nature, and higher on-target efficiency in crop plants (Jaganathan *et al.*, 2018). In summary, these molecular tools, particularly Marker Assisted Breeding and CRISPR can be boon in future drought resistance breeding

### Conclusion

Water is very crucial for survival of life forms on the earth. About 80-85 % of available water is used for agriculture alone. In this challenging era of climate change, it is always advisable to opt for more crop per drop motto to meet the demands of growing population all over the globe. In this regard several approaches were designed to increase WUE in agriculture. In addition, several irrigation methods and agronomic practices have also been employed to avoid water losses. Breeding methods along with molecular tools have emerged as potential avenues in breeding for higher TE or WUE by crop plants. Although many strategies are employed in this regard, it is the duty of each individual to save and use water carefully for both domestic and agriculture purpose.

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Drought being a complex problem, holistic approach of growing drought tolerant cultivars and following appropriate agronomic practices like adjusting the sowing date as per the rainfall pattern of the locality, optimal irrigation at critical stages and choosing water saving irrigation methods helps to prevent water wastage in crop fields.

## References

- 1) Aquib, A. and Nafis, S., 2021. Identifying Meta-QTLs for Stay-Green in Sorghum. *bioRxiv*.
- 2) Azhar, M.T. and Rehman, A., 2018. Overview on effects of water stress on cotton plants and productivity. In *Biochemical, physiological and molecular avenues for combating abiotic stress tolerance in plants* (pp. 297-316). Academic Press.
- 3) Badhan, S., Ball, A.S. and Mantri, N., 2021. First report of CRISPR/Cas9 mediated DNA-free editing of 4CL and RVE7 genes in chickpea protoplasts. *International Journal of Molecular Sciences*, 22(1), p.396.
- 4) Basu, S., Ramegowda, V., Kumar, A. and Pereira, A., 2016. Plant adaptation to drought stress. *F1000Research*, 5.
- 5) Briggs, L. J., and Shantz, H. L., 1913, "The water requirement of plants," in *Bureau of Plant Industry Bulletin* (Washington, DC: US Department of Agriculture), 282–285.
- 6) Cai, Y., Chen, L., Liu, X., Sun, S., Wu, C., Jiang, B., Han, T. and Hou, W., 2015. CRISPR/Cas9-mediated genome editing in soybean hairy roots. *PLoS One*, 10(8), p.e0136064.
- 7) Carriger, S. and Vallée, D., 2007. More crop per drop. *Rice Today*, 6(2), pp.10-13.
- 8) Christopher, J.T., Christopher, M.J., Borrell, A.K., Fletcher, S. and Chenu, K., 2016. Stay-green traits to improve wheat adaptation in well-watered and water-limited environments. *Journal of Experimental Botany*, 67(17), pp.5159-5172.
- 9) Condon, A.G., Richards, R.A., Rebetzke, G.J. and Farquhar, G.D., 2004. Breeding for high water-use efficiency. *Journal of experimental botany*, 55(407), pp.2447-2460.
- 10) Coupel-Ledru, A., Lebon, E., Christophe, A., Gallo, A., Gago, P., Pantin, F., Doligez, A. and Simonneau, T., 2016. Reduced nighttime transpiration is a relevant breeding target for high water-use efficiency in grapevine. *Proceedings of the National Academy of Sciences*, 113(32), pp.8963-8968.

**Comment [IA20]:** References should be listed in the order that they appear in the text. Do not list the references based on the alphabet.

- 11) Dusenre, M.E., Duarte, A.G. and Way, D.A., 2019. Plant carbon metabolism and climate change: elevated CO<sub>2</sub> and temperature impacts on photosynthesis, photorespiration and respiration. *New Phytologist*, 221(1), pp.32-49.
- 12) Fahad, S., Bajwa, A.A., Nazir, U., Anjum, S.A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S. and Ihsan, M.Z., 2017. Crop production under drought and heat stress: plant responses and management options. *Frontiers in plant science*, p.1147.
- 13) Fletcher, A., Christopher, J., Hunter, M., Rebetzke, G. and Chenu, K., 2018. A low-cost method to rapidly and accurately screen for transpiration efficiency in wheat. *Plant methods*, 14(1), pp.1-14.
- 14) Giuliani, R., Koteyeva, N., Voznesenskaya, E., Evans, M.A., Cousins, A.B. and Edwards, G.E., 2013. Coordination of leaf photosynthesis, transpiration, and structural traits in rice and wild relatives (genus *Oryza*). *Plant physiology*, 162(3), pp.1632-1651.
- 15) Hajyzadeh, M., Turktas, M., Khawar, K.M. and Unver, T., 2015. miR408 overexpression causes increased drought tolerance in chickpea. *Gene*, 555(2), pp.186-193.
- 16) Hatfield, J.L. and Dold, C., 2019. Water-use efficiency: advances and challenges in a changing climate. *Frontiers in plant science*, 10, p.103.
- 17) Ilyas, M., Nisar, M., Khan, N., Hazrat, A., Khan, A.H., Hayat, K., Fahad, S., Khan, A. and Ullah, A., 2021. Drought tolerance strategies in plants: a mechanistic approach. *Journal of Plant Growth Regulation*, 40(3), pp.926-944.
- 18) Islam, S.M.F. and Karim, Z., 2019. World's demand for food and water: the consequences of climate change. *Desalination-challenges and opportunities*, pp.1-27.
- 19) Jaganathan, D., Ramasamy, K., Sellamuthu, G., Jayabalan, S. and Venkataraman, G., 2018. CRISPR for crop improvement: an update review. *Frontiers in plant science*, 9, p.985.
- 20) Jury, W.A. and Vaux, H., 2005. The role of science in solving the world's emerging water problems. *Proceedings of the National Academy of Sciences*, 102(44), pp.15715-15720.
- 21) Kamal, N.M., Gorafi, Y.S.A., Abdelrahman, M., Abdellatef, E. and Tsujimoto, H., 2019. Stay-green trait: A prospective approach for yield potential, and drought and heat stress adaptation in globally important cereals. *International Journal of Molecular Sciences*, 20(23), p.5837.

- 22) Khan, A., Pan, X., Najeeb, U., Tan, D.K.Y., Fahad, S., Zahoor, R. and Luo, H., 2018. Coping with drought: stress and adaptive mechanisms, and management through cultural and molecular alternatives in cotton as vital constituents for plant stress resilience and fitness. *Biological research*, 51.
- 23) Kimani, W.M., 2012. *Transferring drought tolerance of the stay-green trait in sorghum from E36-1 an Ethiopian line into Ochuti, a farmer preferred Kenyan variety* (Doctoral dissertation, University of Nairobi, Kenya).
- 24) LI, W., WANG, T., ZHANG, Y. and LI, Y., 2016. Overexpression of soybean miR172c confers tolerance to water deficit and salt stress, but increases ABA sensitivity in transgenic *Arabidopsis thaliana*. *Journal of Experimental Botany*, vol. 67, no. 1, pp. 175- 194. <http://dx.doi.org/10.1093/jxb/erv450>. PMID:26466661.
- 25) Padulosi, S., Thompson, J. and Rudebjer, P.G., 2013. Fighting poverty, hunger and malnutrition with neglected and underutilized species: needs, challenges and the way forward.
- 26) Popkin, B.M., D'Anci, K.E. and Rosenberg, I.H., 2010. Water, hydration, and health. *Nutrition reviews*, 68(8), pp.439-458.
- 27) Prelich, G., 2012. Gene overexpression: uses, mechanisms, and interpretation. *Genetics*, 190(3), pp.841-854.
- 28) Ramamoorthy, P., Lakshmanan, K., Upadhyaya, H.D., Vadez, V. and Varshney, R.K., 2017. Root traits confer grain yield advantages under terminal drought in chickpea (*Cicerarietinum L.*). *Field crops research*, 201, pp.146-161.
- 29) Rehman, A.U., Malhotra, R.S., Bett, K., Tar'An, B., Bueckert, R. and Warkentin, T.D., 2011. Mapping QTL associated with traits affecting grain yield in chickpea (*Cicerarietinum L.*) under terminal drought stress. *Crop Science*, 51(2), pp.450-463.
- 30) Rosero, A., Granda, L., Berdugo-Cely, J.A., Šamajová, O., Šamaj, J. and Cerkal, R., 2020. A dual strategy of breeding for drought tolerance and introducing drought-tolerant, underutilized crops into production systems to enhance their resilience to water deficiency. *Plants*, 9(10), p.1263.
- 31) Ruggiero, A., Punzo, P., Landi, S., Costa, A., Van Oosten, M.J. and Grillo, S., 2017. Improving plant water use efficiency through molecular genetics. *Horticulturae*, 3(2), p.31.
- 32) Sabaghpour, S.H., Mahmodi, A.A., Saeed, A., Kamel, M. and Malhotra, R.S., 2006. Study on chickpea drought tolerance lines under dryland condition of Iran. *Indian Journal of Crop Science*, 1(1and2), pp.70-73.

- 33) Serba, D.D. and Yadav, R.S., 2016. Genomic tools in pearl millet breeding for drought tolerance: status and prospects. *Frontiers in plant science*, 7, p.1724.
- 34) Serraj, R. A., Hash, T. C., Buhariwalla, H. K., Bidinger, F. R., Folkertsma, R. T., Chandra, S., Gaur, P. M. et al. "Marker-assisted breeding for crop drought tolerance at ICRISAT: achievements and prospects." In *Proceedings of the International Congress "In the Wake of the Double Helix: From the Green Revolution to the Gene Revolution"*. Avenue Media, Bologna, Italy, pp. 217-238. 2005.
- 35) Shah, K.K., Modi, B., Pandey, H.P., Subedi, A., Aryal, G., Pandey, M. and Shrestha, J., 2021. Diversified crop rotation: an approach for sustainable agriculture production. *Advances in Agriculture*, 2021.
- 36) Shah, S.M.S. and Ullah, F., 2021. A comprehensive overview of miRNA targeting drought stress resistance in plants. *Brazilian Journal of Biology*, 83.
- 37) Sharma, B., Molden, D. and Cook, S., 2015. *Water use efficiency in agriculture: Measurement, current situation and trends* (No. 612-2016-40604).
- 38) Shavrukov, Y., Kurishbayev, A., Jatayev, S., Shvidchenko, V., Zotova, L., Koekemoer, F., De Groot, S., Soole, K. and Langridge, P., 2017. Early flowering as a drought escape mechanism in plants: How can it aid wheat production?. *Frontiers in plant science*, p.1950.
- 39) Shi, J., Gao, H., Wang, H., Lafitte, H.R., Archibald, R.L., Yang, M., Hakimi, S.M., Mo, H. and Habben, J.E., 2017. ARGOS 8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant biotechnology journal*, 15(2), pp.207-216.
- 40) Shunmugam, A.S., Kannan, U., Jiang, Y., Daba, K.A. and Gorim, L.Y., 2018. Physiology based approaches for breeding of next-generation food legumes. *Plants*, 7(3), p.72.
- 41) Sreeman, S.M., Vijayaraghavareddy, P., Sreevathsa, R., Rajendrareddy, S., Arakesh, S., Bharti, P., Dharmappa, P. and Soolanayakanahally, R., 2018. Introgression of physiological traits for a comprehensive improvement of drought adaptation in crop plants. *Frontiers in chemistry*, 6, p.92.
- 42) Tharanya, M., Kholova, J., Sivasakthi, K., Seghal, D., Hash, C.T., Raj, B., Srivastava, R.K., Baddam, R., Thirunalasundari, T., Yadav, R. and Vadez, V., 2018. Quantitative trait loci (QTLs) for water use and crop production traits co-locate with major QTL for tolerance to water deficit in a fine-mapping population of pearl millet

(Pennisetum glaucum LR Br.). *Theoretical and Applied Genetics*, 131(7), pp.1509-1529.

43) Valavanidis, A. "Blue Planet" Is Expected to Experience Severe Water Shortages? 2019. Available

online: [https://www.researchgate.net/publication/337007636\\_Blue\\_Planet\\_is\\_Expected\\_to\\_Experience\\_Severe\\_Water\\_Shortages\\_How\\_climate\\_change\\_and\\_rising\\_temperatures\\_are\\_threatening\\_the\\_global\\_water\\_cycle\\_on\\_Earth](https://www.researchgate.net/publication/337007636_Blue_Planet_is_Expected_to_Experience_Severe_Water_Shortages_How_climate_change_and_rising_temperatures_are_threatening_the_global_water_cycle_on_Earth)

44) Zhu, T., Ringler, C. and Rosegrant, M.W., 2019. Viewing agricultural water management through a systems analysis lens. *Water Resources Research*, 55(3), pp.1778-1791.

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