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## **Conferring salinity and drought tolerance through overexpression of AVP1, PP2A-C5, and AtNHX1**

Freshwater is a luxury. Without it, irrigation stops and crops fail. Yet with 326 million trillion gallons of water on Earth, this seems like a paradox of sorts. Looking at the numbers, though, the picture becomes more clear. Of these 326 million trillion gallons of water, the vast majority is held in the oceans, in the form of undrinkable saline water. Only 3% is freshwater, of which nearly two-thirds is locked away in ice caps. Thus, less than 1% of water is actually useable for us to drink, bathe, and irrigate with (PSU, 2012). With an ever-growing population, water consumption has exploded at an unsustainable rate. Over the last century, global water consumption has increased by nearly 800 percent (Witze, 2018). Worse yet, fresh water is getting scarcer each year in Somalia where drought has brought widespread crop failures. In order to establish food security in Somalia, it is crucial that we find a viable means of agriculture in this drought-stricken region.

The outlook appears bleak, yet there are viable solutions to this crisis. With desalination plants and massive water diversion projects, Somalia's water needs could be addressed but at a cost simply too high for the developing nation (Forbes 2018). Furthermore, the only body that has the jurisdiction to implement projects such as water desalination plants and water diversion canals is the Somali government. However, any solution to Somalia's drought crisis cannot be contingent on the support of the Somali government due to wide-spread civil conflict. Furthermore, Somalia has little control over its own water supply. Somalia relies on two major freshwater rivers, the Jubba and Shebelle rivers. However, these vital freshwater rivers originate in bordering Ethiopia. Thus, Somalia's does not fully control its major freshwater sources and will thus be severely impacted by the water management policy of Ethiopia. If relations between the two nations deteriorated or Ethiopia saw fit to extract more water, Somalia would face water shortages. Thus, the most viable solution is not to divert or produce more water but instead to utilize existing freshwater more efficiently.

Currently, water conservation efforts have focused on curbing household consumption of water. This is a step in the right direction for more effectively conserving water, but, worldwide, only 12% of freshwater is used for household uses. Industrial processes consume 19% of freshwater; agriculture is by far the largest consumer of freshwater. At first glance, the vast amounts of fresh water that agriculture currently consumes seems like a necessary consequence in order to feed the global population. Yet this is not true. Through smarter irrigation techniques and more drought resistant crop hybrids, Somalia's population could be fed using drastically less water, leaving more water for drinking, bathing, and cooking. In developed regions of the world, this is already the case. In Europe, only 21% of the total freshwater supply is used for agriculture while in countries such as Somalia 82% of all freshwater is used in agriculture (FAO 2016). While conserving in household and industrial water use helps, reducing freshwater use in *agriculture* should be the focus of any water conservation project in Somalia. Thus, this paper focuses on methods to conserve water use in agriculture.

By far, agriculture is the primary economic activity in Somalia. Agriculture employs 80% of all Somalis and accounts for roughly 90% of the nation's exports (World Bank 2010). A typical family in Somalia relies on subsistence farming, growing crops such as maize, sorghum, and rice. Such a family will often have as many as five children and farm on less than 5 hectares. Because of the labor-intensive nature of subsistence farming, the children of the family receive little to no education and stay to help their families; Somalia has one of world's lowest school enrollment, and the total literacy rate sits at 37.8% (Cline 2018).

Furthermore, significant disparities exist in educational attainment. Because Somali families lack the means to send all children to receive education, daughters in Somali families overwhelmingly stay as domestic help. This results in staggering disparities in educational attainment between men and women in Somalia. Men have a literacy rate of approximately 50% while the literacy rate for women sits at 25.8% (Borgen Project). Such disparities educational attainment leads to a generational cycle that perpetuates poverty and inequality. Somalia has a gender inequality index of 0.776 with 1 representing complete inequality (United Nations Development Programme). There is little to no inclusion of Somali women in key government leadership positions. This only further exacerbates gender inequality as Somali policy makers fail to represent Somali women. Thus, the labor-intensive nature of agriculture in Somalia directly affects access to education. By improving the efficiency of agriculture and promoting food security, equal access to education can be improved and, more broadly, Somalia's systematic gender inequality.

Further complicating the issue of food security, Somalia has been a state of constant civil war for the last three decades. This state of constant conflict has left a power vacuum in the region, leading to the appearance of radical groups such as Al-Shabaab. As a result, Somalia has been deemed a fragile state by the Fund for Peace organization (Messner 2014). This constant state of conflict only exacerbates issues of food security as the government diverts resources away from public works projects that could address the issues of water scarcity in irrigation. Moreover, this creates a positive feedback loop wherein the government is unable to aid its citizens resulting further destabilization of the state, which hinders government's capacity to aid its citizens. Because of the fragile state of the Somali government, the solution to the issue of food security cannot rely on the support of the government. Any solution must be a grassroots effort working directly with farmers in Somalia. Thus, this research paper outlines a proposal that would address the issue of food security, without being contingent on the capacity of the Somali government.

With broader shifts in the climate, Somalian families have experienced increasingly common periods of drought, forcing them to utilize ever-increasing amounts of freshwater to irrigate these crops. In East Africa, decades of drought have put more than 11.5 million people at risk of famine, and this number is only forecasted to grow. In the most severely impacted region, Southern Somalia, the crop yield is projected to be less than 50% below the average (NASA 2018).

Without enough rainfall, Somalia could plunge into famine once again. Widespread famine disproportionately harms the most physiologically weak of a community, such as the aged, the children, and the sick (Jelliffe et al. 1992). In Somalia, due to the large number children a family typically has,

these vulnerable groups make up the majority of the population; over 50% of Somalis are under the age of 18 (CIA 2018). As a result, Somali families would be harmed by famine more so than those in other nations.

In Somalia, the specific soil profile is the main factor contributing to agriculture's high consumption of water. Somalia's soil profile is dominated by xerosols, a soil type characterized by its high salinity content (FAO 1974). High soil salinity makes it more difficult for plants to absorb moisture from the soil. Under normal soil conditions, the xylem experiences negative osmotic pressure, allowing roots to uptake water from the soil. However, in high salinity soils, the opposite is true. High osmotic pressure leads to loss of water into the soil (UC ANR 2018). Drought-stressed crops are able to increase their water absorption by uptaking more ions from the soil, yet this presents a new issue. High concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  ions are toxic and detrimental to healthy growth (Tavakkoli et al. 2010). In drought-stricken Somalia, farmers must use what little fresh water they have to irrigate these crops in order to combat high soil salinity, often leaving families with little water for household use.

However, certain crops have methods to mitigate the toxicity of high saline soils. Vacuole accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  ions appears to be utilized by salt resistant grapevine and citrus (Storey et al. 2003). High concentrations of salts can disrupt normal cellular processes, yet by sequestering these ions in the vacuole, plants can ensure that high ion concentrations do not disrupt cellular processes. Thus, genetic engineering efforts to improve drought and salt tolerance have focused on increasing accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  ions into the vacuole. As a result, three genes are aptly suited to enable farmers to grow in Somalia's high salt and drought conditions: PP2A-C5 which mediates vacuolar  $\text{Cl}^-$  sequestration, AtNHX1 which mediates vacuolar  $\text{Na}^+$  sequestration, and AVP1 which mediates the formation of the electrochemical gradient that drives vacuolar ion sequestration.

AVP1, Arabidopsis vacuolar  $\text{H}^+$ -pyrophosphatase gene, is expressed in Arabidopsis thaliana, a small flowering plant found on all continents except Antarctica. Arabidopsis' wide distribution can be attributed to its ability to grow in environments otherwise considered to be inhospitable; it can be found growing in environments as diverse as the Gobi Desert to the US coastal plains. One factor allowing Arabidopsis thaliana to thrive in these diverse environments is the AVP1 gene. The AVP1 gene codes for a vacuolar  $\text{H}^+$ -pyrophosphatase, a proton pump that establishes an electrochemical gradient across the membrane of the vacuole. The proton pump hydrolyzes a diphosphate molecule and results in a net movement of 2  $\text{H}^+$  ions into the vacuole. Overexpression of the AVP1 gene leads to an increase in this electrochemical gradient (Pasapula et al. 2011).

This electrochemical gradient established by the proton pump is crucial in the sequestering of  $\text{Na}^+$  ions into the vacuole because of the next gene: AtNHX1. AtNHX1 encodes a  $\text{Na}^+/\text{H}^+$  vacuole antiporter. The antiporter utilizes the electrochemical gradient established by the proton pump to power the pumping of  $\text{Na}^+$  ions into the vacuole (Sottosanto et al. 2007). In addition, the PP2A-C5 gene has a similar effect as the AtNHX1 gene. Expression of the PP2A-C5 gene mediates sequestration of  $\text{Cl}^-$  anions into the vacuole (Hu et al. 2017). Essentially, the proton pump coded by AVP1 establishes the electrochemical gradient that drives the vacuole  $\text{Na}^+$  sequestration by the antiporter encoded by AtNHX1 while PP2A-C5 gene expression mediates vacuolar  $\text{Cl}^-$  sequestration, thus allowing plants to mitigate  $\text{Na}^+$  and  $\text{Cl}^-$  toxicity.

Previous research has shown that overexpression of AVP1, PP2A-C5, and AtNhx1, by themselves, is enough to increase salt and drought resistance in transgenic crops (Pasapula et al. 2011, Hu et al. 2017, Sottosanto et al. 2007). In addition, co-overexpression of both AVP1 and AtNHX1 as well as co-overexpression of AVP1 and PP2A-C5 has been shown to improve drought and salt tolerance in transgenic crops more than overexpression each of these genes alone (Shen et al. 2014, Li et al. 2018). Considering these genes and the mechanisms through which they confer drought and salinity resistance, there is promise in research into the effects of overexpression of all genes to further improve tolerance against these abiotic stresses.

Thus, the solution is a high impact research project to investigate the potential synergistic effects of overexpression of AVP1, PP2A-C5, and AtNHX1. Funding would be obtained through research grants from government (NSF, USDA, etc.) and industry. This research project presents a radically novel method of improving drought and salinity resistance. Staple crops would be modified through agrobacterium DNA transfer to achieve overexpression of the genes of interest. Transgenic crops would then be identified through the screening of select genetic markers. Such crops would undergo testing to measure their growth in high abiotic stress environments. Factors that would be of particular interest would be total yield, nutritional quality, and resistance to prolonged spans of drought. Secondary aims of the research project would be to identify other genes of interest regarding drought and salinity resistance as well as studying the underlying mechanism behind salinity resistance and the overexpression of AVP1, PP2A-C5, and AtNHX1. All crops that show significant resistance to drought and high saline soils would then be selected for rigorous testing by a third-party organization to ensure the safety of such transgenic crops. Precautionary measures are to be used to prevent gene flow between transgenic and non-transgenic crops.

Following the results of initial findings, these transgenic crops could be introduced into regions such as Somalia where the drought resistance would improve agricultural output in times of drought utilizing existing avenues for humanitarian aid to distribute such transgenic seeds. Humanitarian organizations such as the FAO have conducted previous work with farmers in Somalia. As a result, these organizations are aptly suited for agriculture extension work and for the distribution of these transgenic crops. Such transgenic crops could tolerate higher soil salinity reducing the need for heavy irrigation to mitigate soil salinity. Little to no infrastructure would be required to implement this solution. Furthermore, this solution would be highly cost effective as the production and distribution of transgenic crops can be achieved at a low cost.

While other solutions to address drought are impractical for Somalia, introducing these transgenic crops that co-overexpress AVP1, PP2A-C5, and AtNHX1 could potentially present a low-cost solution to reduce the need for heavy irrigation while improving overall crop output. Such transgenic crops can be prepared at low cost, and once the initial seeds are modified, there is no need for any further genetic modifications. In other words, the changes are hereditary, and future crops grown by Somali farmers would also enjoy the benefits of drought and salt resistance. Such transgenic crops' benefits would be two-part: these crops would reduce the amount of freshwater consumed in irrigation while also ensuring food security, even in periods of drought.

Establishing food security through the implementation of what this paper recommends will have wide-reaching impact. As discussed in this paper, the issue of food security in Somalia is deeply connected to other issues such as violent conflict and disparities in education. A stable and productive agricultural sector is crucial for Somalia's development. By ensuring food security, Somali families can worry less about their next meal, empowering them to pursue a better life.

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