Overexpression of the AtDREB1A gene in almonds with respect to drought tolerance

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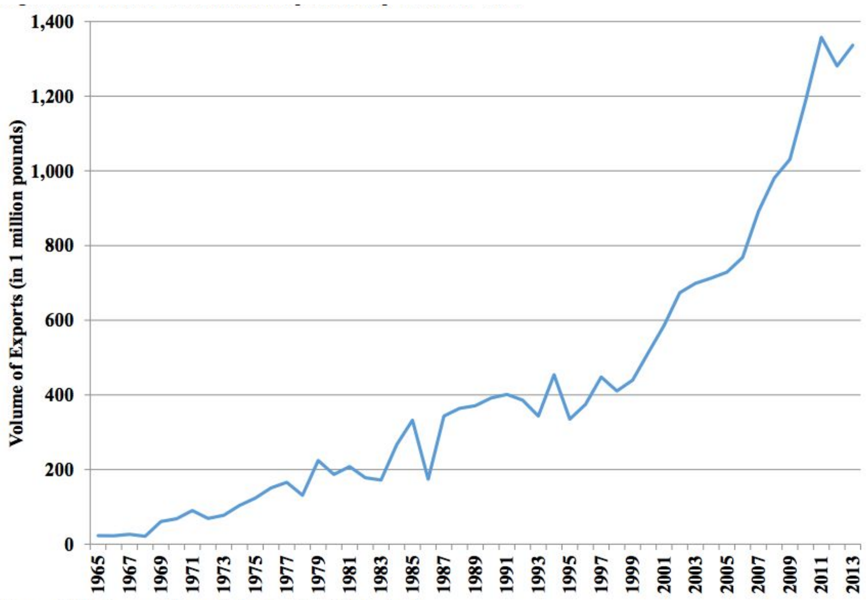
Abstract

One class of transcription factors is the dehydration-responsive-element-binding proteins (DREBs), which can be increased when a plant detects that it is under water deficit conditions. The DREBs, which belong to the group of ethylene responsive factors (ERF), are involved in the regulation of signal transduction pathways under low temperature, salinity and dehydration conditions. One type of DREB is AtDREB1A which has been tested in many different crops, including tomatoes, peanuts, potatoes, and rice. The results have been promising when AtDREB1A was expressed in other crops. Proline contents were increased, ABA levels were kept under normal conditions, and Reactive Oxygen Species levels were decreased. Due to the success of AtDREB1A in other crops, it worth the investment to test the effects AtDREB1A would have in Almonds because the likelihood of success is high.

Introduction

California has the largest economy of any state in the United States and has the 6th largest economy in the world. California is also the largest agricultural producer in the United States and has the most diverse collection of crops compared to any other state. Agriculture represented roughly $47 billion to the states economy in 2015 and currently produces 11.63 percent of total agriculture revenue in the US (Sumner et al., 2013). The top seven revenue producing crops in California in order are almonds, grapes, lettuce, strawberries, tomatoes, walnuts and hay. The export of almonds from California has increased 65 times by volume since 1965, thus making it a vital part of the states economy (Figure 1). Given California's size and it's importance to the United States' and the world's economy, anything that can have a positive impact on the state's economic health is worth pursuing.



Source: USDA Economic Research Service: Tree Nut Yearbook

Figure 1. This shows the volume of almond exports (million pounds) from 1965 to 2013 in the state of California (USDA Economic Research Service, 2013).

Over the past century, California’s almond production has greatly increased and now, the state is responsible for approximately 80% of the world’s almond production (Rankin, 2014). Since the 1990s, almonds have become one of the top crops produced in California's agriculture industry. The huge success of the almond in the Eureka state can primarily be attributed to California's climate which has the ideal characteristics for almond growth: brief cold winters that chill the seed to accelerate the flowering process followed by early warm springs and dry summers. These unique growing conditions make California the only country in North America that produces enough of the nut to export its surplus around the globe. These growing conditions also exist in certain countries in Europe and the Middle East but technological advances allow California to greatly outproduce those nations. In a world where the economy is becoming more global each day, California needs to maximize all of the natural resource advantages that it has in order to find and grow new sources of revenue as well as maximize the sources of revenue that it currently has. Other almond growing countries are also increasing their output and California’s status as the number grower in the world cannot be taken for granted.

California’s annual production of almonds is generally enough to supply the entire United States and still have enough inventory to export to other countries. Annually about 30-40% of California’s almonds are consumed domestically while the rest are imported internationally. If we can increase the yield from almond production, there is an ample world market for our increased inventory. Worldwide, demand for almonds continue to increase with countries like the UK and China having increased their demand for almonds by 45% and 110% respectively since 2012-2013 (Rankin, 2014). Today, India is the world’s largest importer of almonds followed by China and Spain

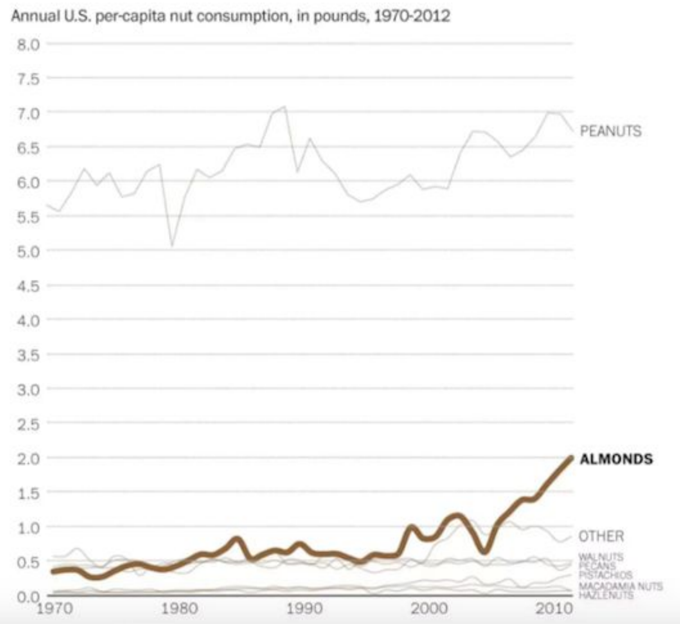


Figure 2. Shows the annual, per capita nut consumption, from 1970-2012 on several different types of nuts

Exportation is not the only incentive to increase our almond production. Americans increased their consumption of almonds by over 220% from 2005 to 2014 (Ferdman, 2014)(Figure 2).

In the early 1970s the average American ate just over a quarter of a pound of almonds annually compared to 2014 where that average raised to over 2 pounds per person. There are a number of reasons for this including the rise of vegetarianism and veganism, the increased desire for a more healthy diet and a trend of people choosing to get their protein from nuts as opposed to red meat.

Another reason that almonds have become increasingly important is the use of almond milk. Almond milk has been a popular substitute to dairy milk for a number of reasons including to accommodate for people who are lactose intolerant, have diabetes, have heart disease or are gluten sensitivities. Almond milk has proven to be more popular than rice or soy milk over time. The global demand for almond milk continues to increase more and more people understand the health benefits as compared to dairy milk. Also, due to the negative impact that the dairy industry has on the environment, it is important to continue to develop more alternatives to dairy use. There are roughly 1.5 billion cows on the planet and their impact has only been a focus in recent years for environmental studies to measure their the numerous negative effects they have on the environment (Collins, 2017).

Almond sales to contribute nearly $11 billion in value to the California economy every year (Sumner et al., 2013). This makes the opportunity to take an already significant portion of California's revenue and increase it without any negative impacts to the environment even more compelling. While the world steadily increasing its demand for almonds, California has the opportunity to refine its production process in order to maximize profits. To facilitate this increase in almond plant efficiency, the almond industry must decrease the growing costs to make them more profitable, and increase the crop yields of almonds so more can be consumed in the US and exported globally.

However, the main factor causing high production costs for almonds is the amount of water they require to grow. In order to grow 1 almond, it takes 1.1 gallons of water, and to grow 1 pound of almonds, it takes around 1900 gallons (Kogon, 2016).

Annually, almonds use about 9.5% of California's agricultural water (Sumner et al., 2013). Due to the dry climate of California, droughts are frequent and water consumption has been a major restraint on the growing industry. According to United States Census Bureau, California's population continues to grow each decade and the growing population puts an even bigger strain on the state's lack of water.

Due to these and other factors, water has become a more expensive commodity, which has raised the prices of crops which heavily on water drastically, thus decreasing profits in the states large agricultural industry. While fresh water is a natural source, it is not a limitless one. The amount of freshwater available for humans to use is largely determined by geographic location. The fact that water is the most expensive ingredient to almond production is a relatively good thing, however. While California is not going to have access to a larger amount of freshwater anytime in the foreseeable future, unless there are drastic climate changes that affect precipitation and runoff, this means that the only way to expand an industry that relies so heavily on water is to use it more efficiently.

One way to decrease the water consumption of almonds is to take advantage of genetic modification and in this regard gene expression, which in this case has enormous potential. Genetic modification of the gene responsible for the drought response systems in the almond itself has the potential to decrease the amount of water the plant needs to survive, which would decrease the amount of natural resources consumed. This could also allow for an increase in the production of almonds, which in turn, increases profits for farmers, as well as the state and helps to create a healthier, more sustainable planetary ecosystem. This increase in production would come with little or no environmental impact, it would simply be an increase in production of an existing crop.

Background

Current environmental stresses, such as heat, salinity, low temperature, drought, and developmental processes, including seed maturation, cause water deficiency in plants. When plants detect a shortage of water, they form reactive oxygen species due to imbalanced water relationship, which changes their physiological processes. This results in decreased plant growth and the overall fruit yield, causing the plant to not reach its maximum yield. In order to combat this water stress response by the plant, the signals it sends that result in stunting fruit growth have to be suppressed.

However, abiotic stress tolerance is a polygenic trait, so developing abiotic stress tolerant plants through traditional breeding approaches can be a challenging task. A large number of transcription factors that are either up- or down-regulated in response to environmental perturbations have been identified using genome-wide transcriptome analyses. An alternative is to genetically engineer plants to introduce stress-tolerance genes, with genes for transcription factors. Transcription factors distinguish certain DNA sequences in regulatory regions and activate downstream genes to respond to abiotic stresses. (Rolla et al., 2013) One class of transcription factors is the dehydration-responsive-element-binding proteins (DREBs), which can be increased when a plant detects that it is under water deficit conditions. Because of the potential of DREBs in regards to abiotic stress response, engineers see a world of potential in them to improve stress tolerance in many plant types.

The DREBs, which belong to the group of ethylene responsive factors (ERF), are involved in the regulation of signal transduction pathways under low temperature, salinity and dehydration conditions (Sarkar et al., 2014). DREB proteins are transcription factors which have a significant role in inducing the expression of many abiotic stress-inducible genes. The DREB or CBF (C-repeat binding factor) family of transcription factors, are a subgroup of the APETALA2(AP2)/ethylene-responsive factor (ERF) family, which is composed of a total of six subgroups (A-1 to A-6)on the basis of their gene structure (Dong et al., 2017).

ABA Acid

Abscisic acid (ABA) is a plant hormone which regulates many physiological processes including: Vegetative development, seed dormancy and germination, and most importantly, the responses to different abiotic stresses like drought and salinity. When a plant is lacking water, the amount of abscisic acid increases in order to regulate the expression of various genes.

In response to water-deficit, plants produce abscisic acid (ABA) which stimulates closure of the stomatal guard cells to reduce water loss. This process generally results in an imbalance between the use and generation of electrons due to decreased availability of CO_2 for photosynthesis (Rai et al., 2013).

ABA is ultimately the central regulator of abiotic stress resistance in plants and coordinates an array of functions, enabling the plants to cope with different stresses. When environmental conditions are harsh, the level of ABA increases via ABA biosynthesis. The increased ABA binds to its receptor to initiate signal transduction leading to cellular responses to stresses therefore, ABA is also called a stress hormone (Sah et al., 2016).

Stomata

Stomata are small pores on the leaf surfaces formed by guard cells, which control plant gas exchange processes. Light usually stimulates stomatal opening whereas ABA and elevated CO_2 levels promote partial or complete closure of stomata. During stomatal closure, decreased gas exchange results in the reduction of photosynthate production while decreased transpiration can reduce water loss from leaves (Suzuki et al., 2013). When a plant is under drought stress, ABA alteration of guard cell ion transport results in stomatal opening, which reduces water loss for the plant.

Reactive Oxygen Species

Reactive oxygen species (ROS) is a generic term used to describe chemical species formed from the incomplete reduction of molecular oxygen. The best-known ROS include superoxide anion (O•-2), hydrogen peroxide (H_2O_2) and the hydroxyl radical (HO•) (Farnese et al., 2016). Reactive oxygen species are critical regulatory molecules for vital processes of cells, produced at low levels, under normal growth conditions. Abiotic stresses alter the cellular homoeostasis and enhance ROS production, which is thought to act as signals for the activation of stress response.

In response to water-deficiency, plants also produce abscisic acid that stimulates closure of the stomatal guard cells to reduce water loss but the problem is this process generally results in an imbalance between the use and the generation of electrons due to decreased availability of CO2 for photosynthesis, leading to overproduction of ROS. Due to highly reactive and toxic nature, the over-production of ROS results in the well-documented phenomenon of oxidative stress (Rai et al., 2013).

These species though can cause considerable damage through peroxidation of membrane lipid components and direct interaction with various macromolecules, causing oxidative damage to chlorophylls, proteins and nucleic acids, making them non-functional.

Proline

In response to different stresses plants accumulate large quantities of different types of compatible solutes. Compatible solutes are low molecular weight, highly soluble organic compounds that are usually non-toxic at high cellular concentrations. One key solute is proline, which provides protection to plants from stress by contributing to cellular osmotic adjustment, ROS detoxification, protection of membrane integrity and enzymes/protein stabilization (Ashraf et al., 2007).

In a stressful environment, proline is overproduced in plants in order to maintain cell turgor/osmotic balance, prevent electrolyte leakage, and making sure the concentration of reactive oxygen species is within normal ranges (Hayat et al., 2012).

AtDREB1A Gene

Crops overexpressing this transcription factor, which is part of the DREB group, has shown to be an efficient way of regulating the expression of abiotic stress-response genes. As more research has been done on the gene, engineers have become more and more interested in its capabilities because of its potential to enhance drought tolerance.

The DRE element contains a 5-bp core sequence of CCGAC, also known as C repeat (CRT), that plays an important role in regulating gene expression in response to low temperature, water deficit, and high salinity (Hsieh et al., 2002). DREB/CBF proteins have a single 60 amino acid-long DNA binding AP2 domain, which permits them to specifically recognize and bind as a single molecule to so-called drought/cold/salt-stress responsive promoter elements (Rolla et al., 2013). The AtDREB1A/CBF3 protein binds to a 5 bp cis-acting core DRE sequence (CCGAC), which is present in the promoter region of many downstream water-deficit stress-inducible genes (Rai, 2013).

Purpose

Currently, almonds require a large amount of water in order to grow, and most of the world's almonds are grown in California. In a state that frequently has droughts and is experiencing rising water costs, it is important to reduce the amount of water consumed by almonds in order to decrease production costs.

To determine the effect AtDREB1A will have on almonds, the effect AtDREB1A has had on other crops drought tolerance must be analyzed. Then it must be concluded whether or not that same effect would occur in almonds.

Research Question

Will the overexpression of the ATDREB1A gene in other plants show a significant change in drought tolerance to offer viable reasoning for further studies in Almonds?

Hypothesis

It is worth the investment to overexpress AtDREB1A in almonds because of reported success in other species.

Null Hypothesis

The effects of overexpressing AtDREB1A in model species indicate little support of applying the treatment to almonds.

Methods

The research design for this project was systematic literature review. Many different peer reviewed papers were used to gather and data. Peer reviewed papers were obtained from Ebscohost, the library database at Cal State Channel Islands University (CSUCI), the online database of University

California at Santa Barbara (UCSB), the National Center for Biotechnical Information (NCBI), and the Almond Board of California. Finding literature concerning the effects of almonds on the California economy was not difficult. As for finding literature relating to AtDREB1A, there were plenty of papers, but many of them did not have data which could be related to other papers data. There were many sources that provided information regarding the use of AtDREB1A in different crops including but not limited to: tomatoes, rice, peanuts, and potatoes. The biggest struggle was that there were only 1-2 papers on each crop, which made it difficult to make sure the data provided was acceptable. There were 2 papers that were very useful in finding data for tomatoes, as well as 2 other papers for peanuts, but for the other crops, finding a second paper could not be done even using university databases.

There was no problem with having papers that were out of date because most of the research regarding AtDREB1A has been done post 2005. This made finding papers easier because it meant there weren't papers that had information which was too old to use or had been disproven.

The crops used for data were peanuts, tomatoes, potatoes, and rice. Data that was obtained about the crops, regarded any of the following: Relative Expression (Fold Increase) of AtDREB1A, Hydrogen Peroxide Concentration, or Proline Content. Although more data was given in papers, that same data could not be found in other papers, which meant it couldn't be accepted.

In order to determine the validity of the data, multiple T-Tests were performed. P values were calculated and based on the P value, the data was either accepted or rejected.

Selection of Criteria

1. Only papers regarding the experimentation of AtDREB1A was used for data collection and analysis.
2. Only papers comparing wild type plants to transgenic plants expressing AtDREB1A were used.
3. Studies that showed the relative expression of AtDREB1A in crops were preferred.

Possible Sources of Error

The main source of error was lack of data replicates which means the data might not be accurate. To correct for this, T-Tests were performed to validate the data.

Results

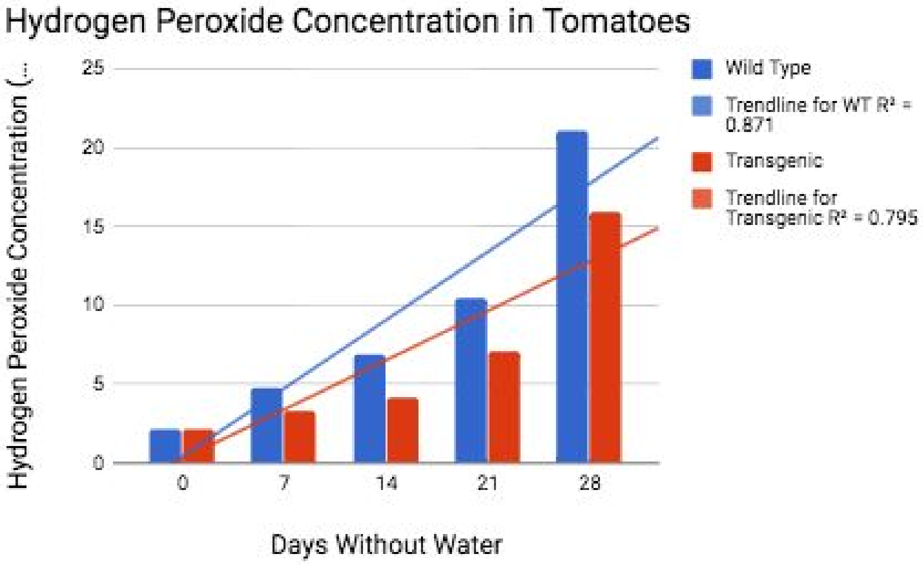


Figure 3A. Displays the Hydrogen Peroxide Concentration (m mol/g) 0 days (well watered), 7 days, 14 days, 21 days, and 28 days after being restricted of water. WT are non-transformed plants while transgenic plants are overexpressing AtDREB1A.

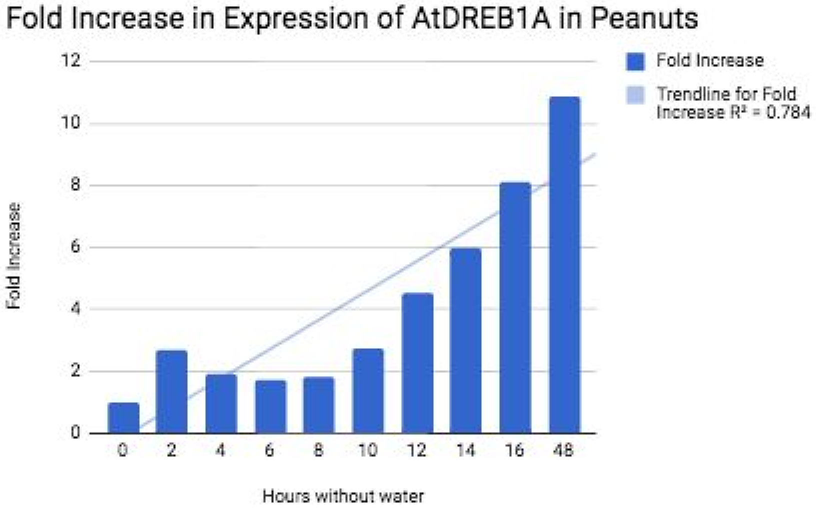


Figure 3B. Displays fold increase of the overexpression of AtDBRB1A in peanuts after 0 (well watered), 2, 4, 6, 8, 10, 12, 14, 16, and 48 hours of being deprived of water.

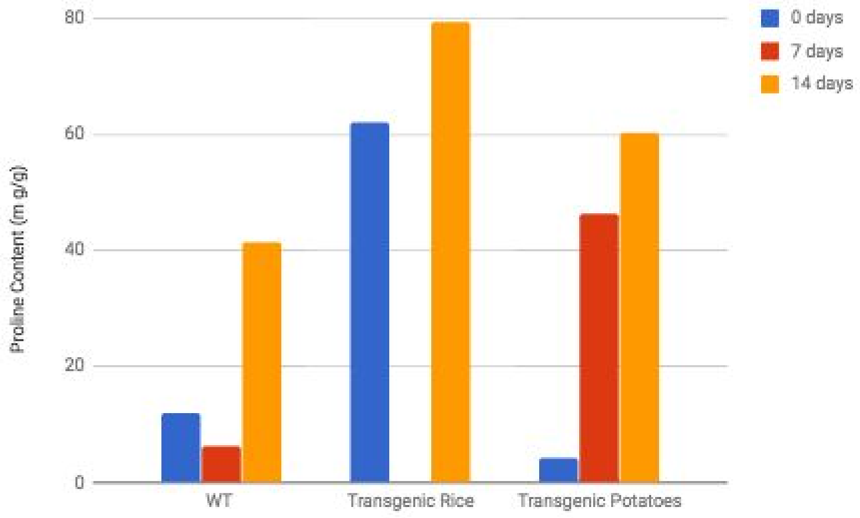


Figure 3C. Displays the proline content (m g/g) in wild type plants, transgenic rice overexpressing AtDREB1A, and transgenic potatoes overexpressing AtDREB1A. Values were taken after 0 (well watered), 7, and 14 days of water deprivation. No value was available for transgenic rice at 7 hours.

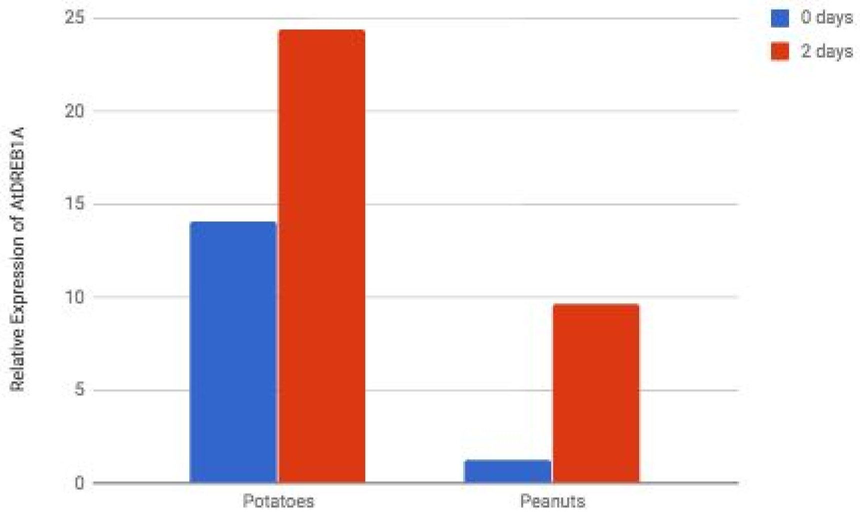


Figure 3D. Displays the Relative Expression (Fold Increase) of AtDREB1A in peanuts and potatoes after 0 days (well watered) and 2 days of being deprived of water.

A total of 4 different crops were analyzed for their effects on drought tolerance. In tomatoes, the hydrogen peroxide concentration steadily increased as the number of days without water increased (Figure 3A). The wild type plants had higher levels of hydrogen peroxide throughout the process compared to the transgenic pants overexpressing AtDREB1A. After performing a T-Test on the data, the P value was found to be 0.000465 making the correlation significant.

In peanuts, the expression of AtDREB1A was found to continually increase as time progressed (Figure 3B). Initially, the expression stayed stagnant, but after a few hours, expression increased significantly. It can be seen that compared to 0 hours (control) expression was much higher at each next time segment. After performing a T-Test on the data, the P value was found to be 0.002 making the correlation significant.

In transgenic rice and transgenic potatoes, the proline content was measured after overexpressing AtDREB1A. Measurements were taken after 0 (well watered), 7, and 14 days of being water deprived. It can be seen that after being deprived of water, proline content in transgenic lines were much higher than wild type. After performing a T-Test on the data, the P value was found to be 0.201 which unfortunately means that the correlation is not significant enough and this data must be excluded until further research is done.

In transgenic potatoes and transgenic peanuts, the relative expression of AtDREB1A was measured after 0 (well watered) and 2 days of water deprivation. The expression of AtDREB1A increased after being subjected to water deprivation. After a T-Test was performed, the P value was found to be 0.221 which means the correlation is not significant enough and is excluded until further data is found.

Discussion

Data analysis of results show that there is a correlation between the overexpression of AtDREB1A and improved drought tolerance. In figure 3A, the hydrogen peroxide concentration was shown to be much lower in transgenic lines as compared to wild type plants. This is significant because although hydrogen peroxide production is good initially for regulating vital processes, overproduction can be detrimental to the plant. The damage they cause to the plant greatly affects the ability of the plant to produce high yields. This means that lowering the hydrogen peroxide concentration in plants is critical to improving crop yields. The overexpression of AtDREB1A was ultimately successful in making tomatoes more drought tolerant because when the plant was under drought stress, the crop yield did not decrease as much as it would have without the overexpression.

In peanuts (Figure 3B), similar results were found. In figure 3B, it is seen that engineers were able to successfully overexpress AtDREB1A. This is important because it shows that overexpression was successful in another crop that is in a different family. Peanuts are in the *Legumes* family, while tomatoes belong to the *Nightshade* family. This means that the transcription factor has been successful in multiple families and is not just limited to one type of family, thus increasing the probability of it being successfully expressed in other families.

Many papers showed there was successful expression of AtDREB1A in potatoes and rice. For rice, it was found that the overexpression of AtDREB1A resulted in an increase in proline production. Proline is extremely important when it comes to drought tolerance because it contributes to cellular osmotic adjustment, ROS detoxification, protection of membrane integrity and enzymes/protein stabilization. When in a water deprived environment, proline is overproduced in plants in order to maintain cell turgor/osmotic balance, prevent electrolyte leakage, and making sure the concentration of reactive oxygen species is within normal ranges. Reactive oxygen species are good in doses, but overproduction damages the plant, which is where proline is very important. Proline makes sure that there is not ROS overproduction. This ultimately saves the plant from taking unnecessary damage. In the end, crop yield is increased because the reactive oxygen species are suppressed and kept within a healthy range. What must be kept in consideration with rice and potatoes is that the data cannot be completely validated until more data is collected. Although the data is not completely valid yet, there are still papers showing the expression of AtDREB1A was successful. Rice is from the *Gramineae* family and potatoes are also from the *Nightshade* family. The successful overexpression in rice though is important because it is yet another family that overexpressed AtDREB1A.

All of this research and data in other crops is important because it adds validity to the hypothesis that almonds will successfully overexpress AtDREB1A. The transcription factor AtDREB1A has proven that it can be effective in multiple different types of crops, even if they are in different families. There's no reason to believe that overexpressing it in almonds, which are in the *Rosaceae* family wouldn't be successful because there have been many experiments done where scientists found extremely positive results.

Ultimately, although limited research has been done, the probability is high that the overexpression of AtDREB1A in almonds would be successful because of its proven track record.

Further Work

There is still much work to be done when it comes to researching the effects of AtDREB1A in different crops. There has been some work done in different crops, but lots of the work needs to be validated. In addition, there aren't many papers that have been published on this topic which leaves a large area of uncertainty open. The effects have been recorded, but when only a few papers have don't the actual experiments, it is hard to confirm the results. Future research should be focused on solidifying the effects that AtDREB1A has on plants when deprived of water. In addition, AtDREB1A should be tested on more crops. The results have been extremely promising, so it should be used on more plants that experience problems with drought. Especially crops grown in California, where droughts are frequent, and water has become an expensive natural resource, it is necessary to start looking for solutions and AtDREB1A is one of those solutions. Ultimately, the area just needs more work done to validate results that have already been found and prove that they are, in fact, true.

Conclusion

California is a state that is constantly getting hit with drought. These droughts greatly increase the cost of water and makes it more and more difficult to obtain.

Most almonds grown in the world, are produced in California and everyday the demand for almonds in increasing. Worldwide, countries are starting to rely on California for their almonds, which has contributed to the states' almond exports increasing drastically over the past decade. As the demand has grown, almonds now find themselves as one of the most valuable crops in California, which makes drought tolerance all the more important. Improving drought tolerance in almonds benefits the state two-fold. First, it decreases the amount of water required to grow almonds, which in turn, reduces the production costs and increases profits. Second, improved drought tolerance is shown to increase crop yield, which means the state will increase the number of almond exports and increase revenue. With an opportunity to make one of California's most valuable crops even more cost efficient, it is important that more experimentation and research be conducted on drought tolerance in almonds.

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