

Exploring the Potential of Transgenic Crops for Improved Fertilizer Use Efficiency

A. Cover Page

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C DFA Funding Request

2012	\$49,531
2013	\$49,724
2014	\$49,923

B. Executive Summary

Crops produced in the desert receive large annual applications of nitrogen (N) and phosphorus (P) fertilizer. However, declining energy supplies and P mineral reserves, erratic fertilizer costs, and concerns about water pollution, has created incentives for improved efficiency. While we have developed management practices such as soil and plant tissue testing and improved fertilizer placement and timing, the possibility of genetic modifications to crops for improved fertilizer use efficiency has received little attention. More recently, it has been shown that over-expression of type I H^+ -pyrophosphatase AVP1 (AVP, *Arabidopsis* vacuolar pyrophosphatase) can enhance nutrient acquisition by crops. AVP1 over-expressing tomato (*Lycopersicon lycopersicum* L.) plants produced more shoot and root biomass than controls when grown under phosphate and nitrate limitations and accumulate more potassium in all conditions tested. Preliminary data we have collected show the potential for yields of AVP1 romaine lettuce (*Lactuca staiva* L.) to be maximized with appreciably less P fertilizer than that required for conventional cultivars. The objective of this project is to evaluate the potential for using AVP1 modified crops for improved nutrient use efficiency under desert cropping systems. In addition to crops we have already modified genetically (potato (*Solanum tuberosum*) and cotton (*Gossypium* spp.), and romaine lettuce), we plan to incorporate the AVP1 trait into iceberg lettuce, a very large recipient of N and P fertilizer. Field studies will be conducted to test control plants and AVP1 modified plants under optimal and suboptimal fertilizer regimes. Fertilizer recoveries and fertilizer use efficiency will be determined and the efficacy of AVP1 modification as a strategy for improved nutrient use efficiency will be evaluated. We aim to demonstrate the economic and environmental benefits of using AVP1 modified crops. We anticipate the successful demonstration of this transgenic technology will ultimately benefit growers whose fertilizer costs are rapidly increasing. In the longer term this project should benefit society at large as natural gas used to produce N fertilizer, and P mineral reserves, are finite resources in decline and air and water pollution associated with fertilization are of continuing concern.

C. Justification

1. Problem

Crops produced in the desert receive large annual applications of nitrogen (N) and phosphorus (P) fertilizers. Amounts of N applied range from 200 to 400 kg/ha and crop recoveries are generally less than 50%. There are numerous possible fates of fertilizer applied N in addition to the desired outcome of crop uptake (Havlin et al., 2005). The urea and ammonium components of the N fertilizer might be lost through ammonia volatilization. The nitrate-N might be lost to leaching with irrigation water below the crop root zone possibly impairing surface and ground water. Nitrate might also be lost as N_2 and N_2O gasses via denitrification processes affecting air quality and climate. Furthermore, all forms of N might be immobilized into the organic soil fraction by the soil microbial population where availability to the crop is delayed. Nitrogen fertilizer production depends on natural gas availability and prices.

Amounts of P applied to crop production systems often approach and exceed 200 kg P/ha and crop recoveries of P fertilizers are generally less than 20%. While much of the added P is converted to insoluble forms in the calcareous soils of the region (Sanchez, 2007), some of it is potentially carried off in runoff and drainage water into receiving surface waters having adverse ecological effects (Izuno et al., 1991). Further, erratic fertilizer pricing over the past several years has created incentives for improved efficiency. Approximately three years ago, the costs of mono-ammonium phosphate (MAP), a formulation widely used for desert vegetable production, exceeded \$1,200.0 per ton. Although costs have since declined, rapid increases are anticipated as the world economy recovers and resource demand in the developing world regains momentum. In addition, world P reserves are rapidly declining and there is concern that a shortage of P fertilizers will ultimately compromise world food production (Vaccari, 2009).

Over the past two decades, researchers with the Universities of Arizona and California have developed strategies for efficient nutrient management. For N, these practices include fertilizer timing or controlled release fertilizers, pre-sidedress plant and soil testing, and improved irrigation management (Sanchez and Doerge, 1999). For P, these practices include soil test based fertilizer recommendations and exploitation of innovative placement technologies (Sanchez, 2007). However, the possibility of genetic modifications to commercial crops for improved fertilizer use efficiency has received little attention. A high fertilizer use crop such as lettuce shows very little variation in response to fertilizer, among commercial cultivars currently used (Nagata et al., 1992; Sanchez et al., 1995).

More recently, it has been shown that that over-expression of type I H^+ -pyrophosphatase AVP1 (AVP, *Arabidopsis* vacuolar pyrophosphatase) contribute positively to many energetic plant processes including general growth, nutrient acquisition, and stress response (Yang et al., 2007). This genetic modification enhances nutrient uptake by affecting the abundance and activity of the plasma membrane H^+ -ATPase in a manner that correlates with apoplasmic pH alterations and rhizosphere acidification. Rhizosphere acidification is a central mechanism for plant mineral nutrition since it contributes to nutrient solubility and the plasma membrane proton motive force. Preliminary data we have collected show the potential for yields of AVP1 romaine lettuce to be maximized with less P fertilizer than that required for conventional cultivars.

The objective of this project is to evaluate the potential for using AVP1 modified plants for improved nutrient use efficiency under desert cropping systems. Studies will include potato, lettuce, and cotton. Field studies will include N and P rate studies and comparisons of AVP1 modified cultivars and conventional cultivar counterparts. These studies will be conducted at university research centers in Yuma and Maricopa AZ, and in Holtville and Thermal, CA due to existing restrictions concerning GMOs and field production. Additionally, we seek to genetically modify iceberg lettuce for AVP1 expression and perform preliminary evaluations of these modifications.

2. CDFA/FREP Goals

This project is consistent with the 2011 CDFA/FREP goal of “Devising innovative techniques to improve fertilizer use efficiency”.

3. Impact

This project should have an impact in the entire western United States where achieving efficient N and P fertilization practices remains a continuing challenge. This region includes a number of high value crops that receive large amounts of N and P fertilizer. This project includes evaluations of moderate (cotton), high (potato), and very high (lettuce) fertilizer using crops.

4. Long Term Solutions

This project will provide a preliminary data base toward utilization of the AVP1 transgenic technology as a long term solution to sustainable fertilizer management.

5. Related Research

It has been recognized for some time that modifications of root biomass and architecture is a potential strategy for improving water and nutrient utilization by cultivated crops (Jackson, 1995; Gallardo et al., 1996). Wild relatives of cultivated crops often seem to have roots systems that can better exploit soil water and nutrient resources (Chapin et al., 1989) but progress in incorporating desirable root characteristics from wild plants into cultivated relatives has been limited (Johnson et al, 2000).

More recently, it has been demonstrated that overexpression of AVP1 resulted in plants with increased root and shoot biomass compared to conventional counterparts. Further analysis showed that leaf enlargement in AtAVP1-OX plants results from increased cell numbers (Li et al., 2005; Gonzalez et al., 2010), and the observed 1.6 to 8.4-fold increase in root growth likely does as well (Li et al, 2005). Of note, engineering a 35S:AVPID cassette into rice plants resulted in 82% and 109%

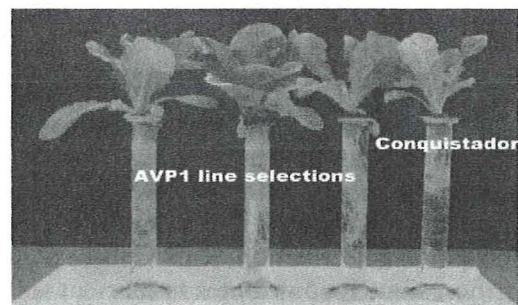


Figure 1. Shoot and root growth of AVP1 lines and a conventional romaine lettuce.

enhancement of shoot and root biomass, respectively. These observed increases are not specific to AVP1 and Arabidopsis since additional studies have shown that overexpressing orthologs *TsVP* from *Theilungiella halophila* (Lv et al., 2008; 2009; Li et al., 2010) and *TVPI* from wheat (Brini et al., 2007) also effectively increase biomass in other plant species. In cotton overexpressing *TsVP*, the photosynthesis rate, stomatal conductance and root development were higher than controls under non-stress conditions, whereas these parameters were significantly lower than controls in antisense *TsVP* plants (Lv et al., 2008). In an elite inbred corn line, overexpressing *TsVP* resulted in 21% higher accumulation of total soluble sugars and enhanced root development. These differences were elevated to ~50% after three days of osmotic stress treatment and the 1000-grain weight from plants subjected to six-weeks drought stress was 30% higher than controls (Li et al., 2008). Of note, in an independent experiment conducted by Gaxiola and colleagues, it was shown that 35S:AVP1 cotton plants developed larger root systems and under dry-land conditions and fiber yield was at least 20% higher than that of controls (Pasapula et al., 2010).

In addition to effect on root biomass, AVP1 over-expression also enhances nutrient uptake by affecting the abundance and activity of the PM H^+ -ATPase in a manner that correlates with apoplastic pH alterations and rhizosphere acidification (Li, 2005; Yang, 2007). Rhizosphere

acidification is a central mechanism for plant mineral nutrition since it contributes to nutrient solubility and the PM proton motive force (pmf). Consequently, AVP1 overexpressing Arabidopsis, tomato and rice plants outperform controls when grown under phosphate limitations and accumulate more potassium in all conditions tested (Yang, 2007; Gaxiola, 2011). Interestingly, only AVP1 transgenic tomato produced healthy fruits in limiting Pi conditions (Yang, 2007). Furthermore, up-regulation of AVP1 in Arabidopsis and *Lactuca sativa* (LsAVP1- OX) resulted in larger roots and shoots than controls when grown with limiting nitrate (1.5 mM). The root acidification capacity of these LsAVP1-OX plants appears to be responsible for their capacity to thrive in limiting nitrate or phosphate conditions (Gaxiola unpublished data).

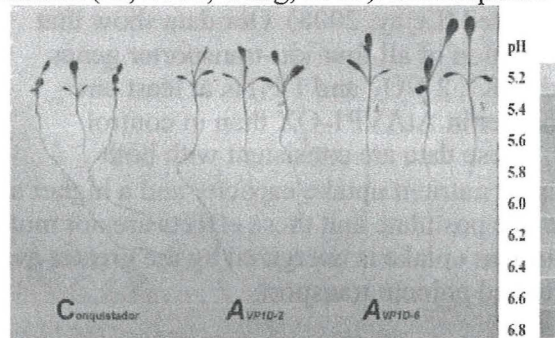


Figure 2. Root growth and rhizosphere acidification of conventional and AVP1 transgenic lettuce

Immunohistochemical data on AVP1 over-expressing transgenic Arabidopsis (Gaxiola, 2001), cotton (Pasapula, 2010), and lettuce (Gaxiola, unpublished data) are consistent with an enhanced H^+ -PPase abundance at the vasculature of leaves. Interestingly, early immunolocalization studies showed that the H^+ -PPase and PM H^+ -ATPase localize in close proximity at the plasma membrane (PM) of the sieve elements in *R. communis* (Langhans, 2001; Long, 1995; Robinson, 1996). Furthermore, we have recently shown via double epifluorescence and immunogold labeling experiments that the co-localization of AVP1 and PIP1 (*a bona fide* PM maker) in PM of sieve element-companion cell complexes from *Arabidopsis thaliana* (Gaxiola, unpublished data). Since AVP1-OX plants have increased H^+ -PPase expression in the phloem, and photo-assimilates partition has been recognized as a major determinant of crop yield (Giaquinta, 1983), we measured the rates of sugar exudation from cut stems of 24 d-old rosettes using an

established EDTA-exudation method (Srivastava, 2008). As expected, glucose and fructose levels were not different in conventional and AVP1-OX exudates. However, sucrose exudation rates from AVP1-OX rosettes were nearly double that of unmodified plants. As an alternative test for enhanced sucrose transport to sink organs, source leaves were photosynthetically labeled with $^{14}\text{CO}_2$ and ^{14}C transport into roots measured by autoradiography and phosphor-imaging. AVP1-OX roots accumulated more ^{14}C , and this was concentrated in the growing regions of the main and lateral roots. The results from exudation experiments and from transport studies with whole plants are therefore corroborative and support our hypothesis that phloem transport is enhanced.

One of the most remarkable traits associated with the up-regulation of AVP1 is an enhanced capacity for nutrient uptake. Regulation of the root K^+ , NO_3^- and P_i transporter genes coordinate with photo-synthesis and the carbon status of the plant (Lejay, 2008). We monitored the expression of four root ion transporters whose sugar induction has been extensively documented (Lejay, 2008). Our data show that the induction of all four ion-transporter genes tested (1 K^+ , 2 NO_3^- and 1 P_i) is at least one fold higher in AtAVP1-OX than in control roots. These data are consistent with both enhanced nutrient uptake capacity and a higher availability of reduced carbon in AVP1-OX roots. We postulate that these effects are not mutually exclusive, but that the enhanced capacity for nutrient uptake is energized by the greater availability of sucrose brought about by augmented phloem transport.

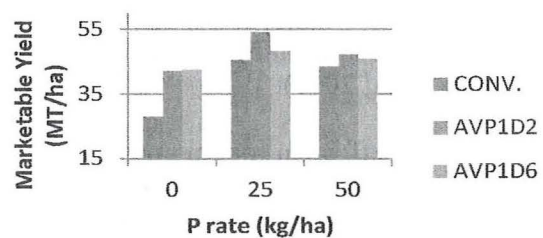


Figure 3. Response of conventional and AVP1 modified romaine lettuce to P on a soil testing 25 ppm pre-plant Olsen P. Note that soils testing less than 20 ppm are infrequent in the Yuma Valley and conventional lettuce will respond to P fertilizer up to a soil test P level of 35 ppm.

Much less has been done on potential applications of this technology as a tool for improved nutrient use efficiency in commercial production settings. In preliminary studies we conducted in 2010-2011 we found AVP1 modified romaine lettuce was able to take up more P from low P testing soils compared to unmodified counterparts (unpublished greenhouse data of Sanchez and Gaxiola). Yields of AVP1 modified romaine lettuce were higher at lower P rates compared to a conventional counterpart. Further one field study, where the soil tested 25 ppm Olsen P pre-plant, AVP1 modified romaine lettuce showed significantly greater yields compared to a conventional romaine when no P was added.

6. Contribution to knowledge base

We aim to show that AVP1 transformation of crops is a viable strategy toward achieving enhanced nutrient use efficiency in real world production settings. This will ultimately result in more economically viable, more environmentally friendly, and more sustainable crop production.

7. Grower use

Growers are anxious about rapidly increasing fertilizer costs. It is anticipated that fertilizer prices will continue to increase as resource demand in the developing world continues to increase and natural gas and P mineral reserves continue to decline. If this technology becomes available the adaptation will be driven by policy and economic considerations.

Perhaps the greater existing obstacle to adaptation is public concerns about genetically modified food crops and resulting policy. However, we believe once the economic and environmental benefits of this technology are proven, and the gravity of looming resource shortages appreciated, the use of this transgenic modification of crops will be accepted as part of a comprehensive strategy for sustainable agriculture.

D. Objectives.

The objectives of this project are to evaluate and demonstrate the potential for using AVP1 modified plants for improved nutrient use efficiency under desert cropping systems. Studies will include potato, lettuce, and cotton. We have already modified potato, cotton and romaine lettuce. As part of this project we seek to modify iceberg lettuce, a significant user of fertilizer in the western United States.

E. Work plan and methods

Task 1 (January 2012 through December 2012). Make AVP1 modification to two cultivars of iceberg lettuce. We will modify a late fall and midwinter iceberg lettuce cultivar. Please note we have already modified potato, cotton, and romaine lettuce.

Enhanced *AVP1* expression has been associated with improved yield in a number of crops (Yang, 2007, **Fig. 3**). We will further examine the effects of AVP1 expression on yield performance by transforming two cultivars of iceberg lettuce (one late fall and a midwinter lettuce cultivar) with the 35SAVP1 expression cassette.

Subtask 1a (January 2012 to March 2012).

Plant transformation will be carried at the Ralph M. Parsons Plant Transformation Facility at UC Davis. We will provide the genetically engineered *Agrobacterium* strain carrying the 35S:AVP1 construct and for this we will obtain a USDA-APHIS permit for interstate shipments of genetically engineered *Agrobacterium*.

Subtask 1b (March 2012 to June 2012).

Plant received from contractor will be screened using phenotypic screening techniques and PCR procedures. This work will be done at the Cellular and Molecular Biosciences Laboratory at ASU. We have shown that a characteristic phenotype of AVP1-OX plants have an enhanced rhizosphere acidification capacity (**Fig. 2**). This phenotype is accentuated if the plants are subjected to nutrient limitation stress (Yang, 2007). We will monitor this response in seeds of the PCR positive plants generated at the plant transformation facility. This simple test will help to establish if there is a differential root acidification capacity in the genetically modified lettuce

and if this capacity correlates with AVP1 expression levels and/or phloem sucrose levels. The AVP1 protein expression levels will be also monitored on the best candidates.

Subtask 1c (July 2012 to November 2012).

Once suitable lines are identified seed will be increased under field and greenhouse conditions at the Yuma Agricultural Center.

Task 2 (January 2012 to September 2012). Evaluate response of conventional and AVP1 modified cultivars of potato and cotton to N and P fertilizer rates.

Task 2a (January 2012 to June 2012). Conventional and AVP1 modified potatoes will be seeded in N and P fertilizer rate studies. The N rates will be 0, 50, 100, 150, and 200 kg N/ha. The N will be applied in three split applications. The P rates selected will depend on pre-plant soil tests and will likely range from 0 to 150 kg P/ha all applied pre-plant. The experiments will be randomized complete block with four replications. Petioles will be collected from all plots through the season, oven dried, and ground to determine crop nutritional status. Nitrate-N will be determined potentiometrically after extraction with $\text{Al}_2(\text{SO}_4)_3$ buffer solution (Baker and Smith, 1969). Phosphate-P will be determined colorimetrically after extraction with 2% acetic acid. Soil samples will be collected from all plots and P will be measured colorimetrically after sodium bicarbonate extraction (Olsen et al., 1954). At harvest, marketable yield and total N and P accumulation will be determined. We will collect four whole above ground plants from each plot which will be shredded, oven dried, and ground. The ground tissue will be analyzed for total N using combustion spectroscopy. Phosphorus concentrations in the ground tissue will be determined by ICP/MS after digestion with peroxide and sulfuric acid. Data will be subjected to analysis of variance using an appropriate statistical model. These studies will be performed at the Yuma Agricultural Center (Mesa farm site). We do have soils testing low in P on the Yuma Mesa site. It is important we select sites testing low to medium (5 to 15 ppm Olsen P) to evaluate the full potential of this genetic modification.

Task 2b (March 2012 to September 2012). Conventional and AVP1 modified cotton will be seeded in N and P fertilizer rate studies. The N rates will be 0, 50, 100, 150, and 200 kg N/ha. The P rates selected will depend on pre-plant soil test and will likely range from 0 to 75 kg P/ha. The experiments will be randomized complete block with four replications. N and P nutritional status will be monitored by season long tissue test as described above. Marketable yield and total N and P recovery to fertilizer rate will be determined at maturity as described above. Data will be subjected to analysis of variance using an appropriate statistical model. These studies will be performed at the Maricopa Agricultural Center due to generally lower observed soil test P levels (generally 5 to 10 ppm Olsen P). Following years of vegetable production soil test P levels at the Yuma Agricultural Center Valley Farm are medium to high (usually >20 ppm Olsen P) and the soils on the Yuma Mesa farm are considered too light (coarse) for cotton production.

Task 3 (November 2012 to April 2013) Evaluate response of conventional and AVP1 modified cultivars of romaine lettuce to N and P fertilizer rates.

Task 3a Conventional and AVP1 modified romaine lettuce will be seeded in N and P fertilizer rate studies. The N rates will be 0, 50, 100, 150, and 200 kg N/ha. The N will be applied in three

split applications. The P rates selected will depend on pre-plant soil tests and will likely range from 0 to 200 kg P/ha all applied pre-plant. The experiments will be randomized complete block with four replications. N and P nutritional status will be monitored by season long tissue test as described above. Marketable yield and total N and P recovery to fertilizer rate will be determined at maturity as described above. Data will be subjected to analysis of variance using an appropriate statistical model. These studies will be performed at the Maricopa Agricultural Center due to the presence of low P testing soils.

Task 4 (January 2013 to September 2013). Second field evaluation of response of conventional and AVP1 modified cultivars of potato and cotton to N and P fertilizer rates.

Task 4a (January 2013 to June 2013). Conventional and AVP1 modified potatoes will be seeded in N and P fertilizer rate studies. The N rates will be 0, 50, 100, 150, and 200 kg N/ha. The N will be applied in three split applications. The P rates selected will depend on pre-plant soil test and will likely range from 0 to 150 kg P/ha all applied pre-plant. The experiments will be randomized complete block with four replications. N and P nutritional status will be monitored by season long tissue test as described above. Marketable yield and total N and P recovery to fertilizer rate will be determined at maturity. Data will be subjected to analysis of variance using an appropriate statistical model. These studies will be performed at the UCR Research Center at Thermal, CA. We will locate our site based on pre-plant soil tests. In this study we will use resin samplers to estimate N leaching losses using methods described by others (Li et al., 1993). Briefly these systems consist of 3 cm diameter PVC access tubes installed at a 60° angle so that 2 cm PVC tubes fitted with a resin can be installed or retrieved at desired sampling intervals. The sampling systems are installed at an angle so that the soil layers above the resin are undisturbed. The resin will be located approximately 50 cm below the soil surface. We anticipate we will retrieve resins after every other irrigation event. These resins will be extracted by sequential shaking with 2 N KCl and the resulting extracts will be composited for ammonium and nitrate analysis. Total ammonium and nitrate N in the resin extracts will be determined colorimetrically. Ammonium will be determined using the indophenol blue method, and nitrate will be determined using Griess-Ilosovay method after reduction with copperized cadmium (Mulvaney, 1996). These both were measured using an ALPKEM RFA2 automated colorimeter. We will use these systems to obtain qualitative estimates of N leaching from all plots.

Task 4b (March 2013 to September 2013). Conventional and AVP1 modified cotton will be seeded in N and P fertilizer rate studies. The N rates will be 0, 50, 100, 150, and 200 kg N/ha. The P rates selected will depend on pre-plant soil test and will likely range from 0 to 75 kg P/ha. The experiments will be randomized complete block with four replications. N and P nutritional status will be monitored by season long tissue test as described above. Marketable yield and total N and P recovery to fertilizer rate will be measured at maturity. Data will be subjected to analysis of variance using an appropriate statistical model. These studies will be performed at the Maricopa Agricultural Center due to generally low observed soil test P levels.

Task 5 (October 2013 to February 2014). Field evaluation of response of conventional and AVP1 modified cultivars of iceberg lettuce to N and P fertilizer rates.

Subtasks 5a and 5b. Conventional and AVP1 modified iceberg will be seeded in N and P fertilizer rate studies. The N rates will be 0, 50, 100, 150, and 200 kg N/ha. The N will be applied in three split applications. The P rates selected will depend on pre-plant soil tests and will likely range from 0 to 200 kg P/ha all applied pre-plant. The experiments will be randomized complete block with four replications. N and P nutritional status will be monitored by season long tissue test. Marketable yield and total N and P recoveries to N and P rate will be determined at maturity. Data will be subjected to analysis of variance using an appropriate statistical model. The fall cultivar will be seeded in September and the winter cultivar in November. These studies will be performed at the Maricopa Agricultural Center due to generally low observed soil test P levels. In this study we will use the resin samplers methodology described above to estimate N leaching losses.

Task 6 (September 15 2014 to December 30, 2014). Field evaluation of response of conventional and AVP1 modified cultivars of iceberg lettuce to irrigation and N fertilization and P fertilization.

Subtasks 6.1 It is well known that irrigation affects crop N responses (Sanchez, 2000) and preliminary evidence we have suggested AVP1 modification affects crop response to water as well as fertilizer. Thus, this study will include a factorial combination of irrigation and N fertilization using drip irrigation on the Yuma Mesa Research farm. A manifold design will include 3 N regimes (75, 150, 225 kg N/ha) and three irrigation regimes (0.75, 1.00 and 1.25 ET^o). N nutritional status will be monitored by season long tissue test. Marketable yield and total N and recoveries to N rate will be determined at maturity. Marketable yield and total N recoveries to N and rate will be determined at maturity. In this study we will use the resin samplers methodology described above to estimate N leaching losses. Data will be subjected to analysis of variance using an appropriate statistical model.

Subtask 6.2 This is a simple P rate study with iceberg lettuce. The P rates selected will depend on pre-plant soil test and will likely range from 0 to 200 kg P/ha all applied pre-plant. The experiments will be randomized complete block with four replications. P nutritional status will be monitored by season long tissue test. Marketable yield and total P recoveries to P rate will be determined at maturity. Data will be subjected to analysis of variance using an appropriate statistical model. This study will be performed at the UCD Desert Research Center at Holtville, CA.

F. Project Management, Evaluation, and Outreach

1. Management

The project will be directed overall by Dr. Sanchez of the Univeristy of Arizona's Yuma Agricultural Center. Genetic screening and validations will be performed by Dr. Roberto Gaxiola at the Cellular and Molecular Biosciences Laboratory at ASU. All field evaluations will be directed by Dr. Sanchez.

2. Evaluation

During this project we will collect data to demonstrate the economic and environmental benefits of this technology. These data should ultimately aid in scientific based

discussions concerning the potential risk and rewards of the transgenic technology utilized. Furthermore, direct benefits of these technologies to the agricultural community will be demonstrated in outreach activities.

Planned Outreach activities

March 2012	Field Day at Southwest Agricultural Conference (Yuma, AZ)
August 2012	Presentation at Fall Vegetable Workshop (Yuma AZ)
November 2012	Presentation at Winter Desert Workshop (Holtville, CA)
January 2013	Presentation at grower meeting in Coachella Valley (Indio, CA)
March 2013	Presentation at Southwest Agricultural Conference (Yuma AZ)
May 2013	Presentation at Desert Agricultural Conference (Casa Grande, AZ))
November 2013	Presentation at Winter Desert Workshop (Brawley, CA)
January 2014	Presentation at Extension meeting in Parker CA.
March 2014	Presentation at Southwest Agricultural Conference (Yuma AZ)
November 2014	Field day at Desert Agricultural Center in Holtville (Holtville, CA)

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