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Summary report on the 2013-NPB project "Creation and Analysis of Drought-, Heat-, and Salt-Tolerant Peanuts"

PIs: Hong Zhang and Paxton Payton

To make peanut significantly more tolerant to drought, heat and salt stresses, we proposed to create and analyze transgenic peanut plants that simultaneously express two genes, OsSIZ1 and AVP1. Overexpression of these two genes in peanut will likely significantly improve tolerance to drought, heat and salt stresses, which will have a major positive impact on the peanut industry in West Texas. The proposed research addresses the goal of National Peanut Board to increase peanut yield and to make peanut production profitable in water-limited West Texas.

Without continuing support from NPB or TPPB, this project will have to come to an end. We will terminate this project at the end of August, 2014. In summary, we have been able to show that it is possible to make peanut significantly more tolerant to salt, drought, or to both salt and drought simultaneously by overexpressing foreign genes such as AVP1, AtNHX1, and IPT (Qin et al., 2011 and 2013; Banjara et al., 2012). We anticipate that as climate change brings dramatic changes in agricultural production in the US, especially in West Texas, there will be a need for creating heat-, drought- and salt tolerant crops including peanut. We are one step ahead in preparing peanut for the unavoidable outcome of climate change.

We thank NPB for supporting our research on peanut at Texas Tech University!

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Final report on the 2013-NPB project "Creation and Analysis of Drought-, Heat-, and Salt-Tolerant Peanuts"

PIs: Hong Zhang and Paxton Payton

Goal of the project: **To make peanut significantly more tolerant to drought, heat and salt stresses, we proposed to create and analyze transgenic peanut plants that simultaneously express two genes, *OsSIZ1* and *AVP1*. Overexpression of these two genes in peanut will likely significantly improve tolerance to drought, heat and salt stresses, which will have a major positive impact on the peanut industry in West Texas. The proposed research addresses the goal of National Peanut Board to increase peanut yield and to make peanut production profitable in water-limited West Texas.**

Rationale of this project

With previous supports from NPB and TPPB, we were able to create transgenic peanut plants that express the *AVP1* gene (Qin et al., 2013). The *AVP1*-transgenic peanut plants displayed increased salt tolerance (Fig. 1A) and drought tolerance (Fig. 1B) in greenhouse. In the field conditions, *AVP1*-transgenic peanut plants produced higher yield than wild-type peanut plants. Our collaborator Dr. Hong Luo's group demonstrated that heterologous expression of OsSIZ1, a SUMO E3 ligase from rice, could dramatically improve heat and drought tolerance in transgenic bentgrass (Li et al., 2013). Therefore, we believed that overexpressing of *AVP1* and *OsSIZ1* in peanut will likely make transgenic peanut plants simultaneously more tolerant to heat, drought, and salt stresses.

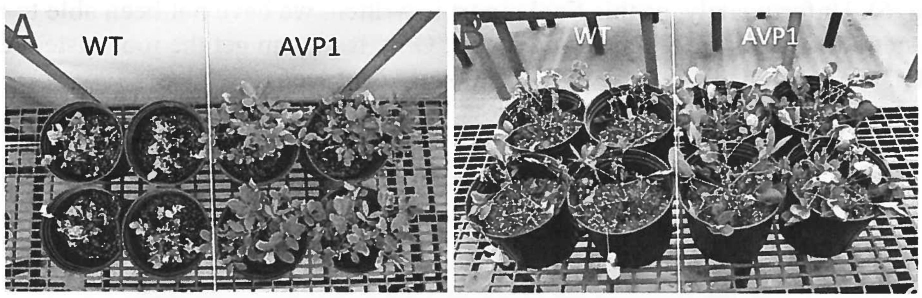

Fig. 1. *AVP1*-transgenic peanut plants display significantly improved performance under salt and drought conditions in greenhouse. **A.** Salt tolerance test. Plants were treated with 200 mM NaCl for three weeks. **B.** Drought tolerance test. Plants were treated with reduced irrigation conditions for 4 weeks. WT, wild-type peanut plants; AVP1, transgenic peanut plants.

Approach

1. Construction of transformation vectors

We first made two transformation vectors, *AVP1/OsSIZ1* and *AVP1/OsSIZ1/AtNHX1*, respectively (Fig. 2). The first vector contains two genes, *AVP1* and *OsSIZ1*, which is expected to confer increased heat-, drought-, and salt-tolerance if both genes are overexpressed in transgenic peanut plants. The second vector contains three genes, *AVP1*, *OsSIZ1*, and *AtNHX1*, which is expected to increased confer heat- and drought-tolerance, and confer even higher salt-tolerance if three genes are overexpressed in transgenic peanut plants (for the proof of concept in using *AVP1* and *AtNHX1* to improve drought and salt tolerance in peanut, please see our published papers: Banjara et al., 2012 and Qin et al., 2013).

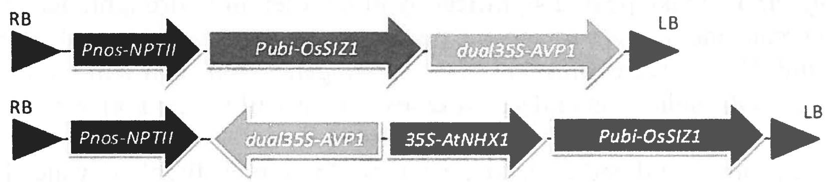

Fig. 2. Transforming vectors for peanut transformation. RB and LB, right border and left border of T-DNA. *Pnos*, nopaline synthase promoter; *NPTII*, kanamycin resistance gene; *Pubi*, ubiquitin promoter from maize; *OsSIZ1*, the rice SIZ1 gene; *dual35S*, dual 35S promoter.

2. Peanut transformation

We used the Agrobacterium-mediated transformation method (Sharma and Anjaiah, 2000) to introduce the two constructs into peanut.

3. Go-going research

We started peanut transformation by infecting peanut with Agrobacteria containing the transforming vectors shown in Fig. 2. Then we obtained hundreds of putatively transformed, first calli (Fig. 3), regenerating shoots (Fig. 4), regenerating plants (Fig. 5), and bigger regenerating shoots (Fig. 6). Unfortunately, as this final report is written, we have not been able to regenerate the root system for any of the regenerating plants. Only if we can get the root system established in the media, can we transplant these plants into soil to get seeds.

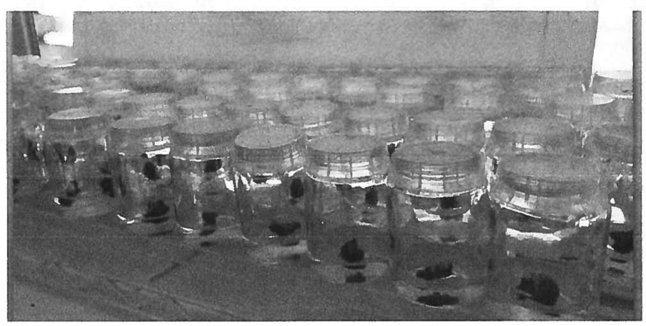

Fig. 3. Putative transgenic calli were generated after Agrobacteria infections.

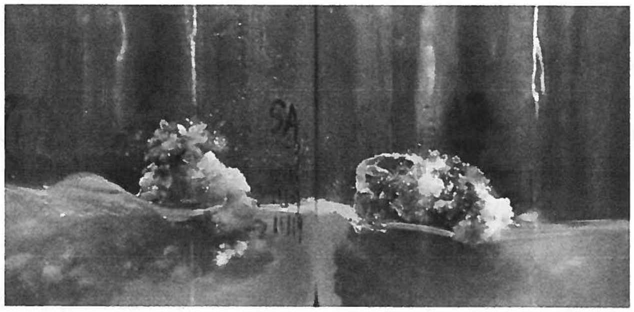

Fig. 4. Putatively transformed shoots are being regenerated.

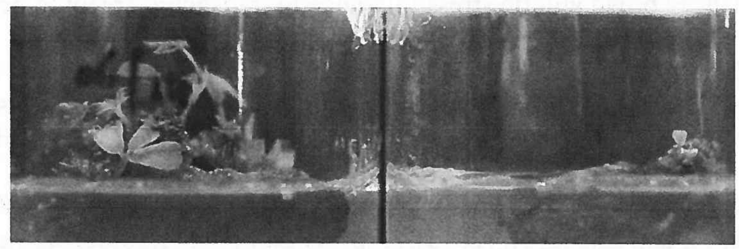

Fig. 5. Putatively transformed peanut plants are growing in media.

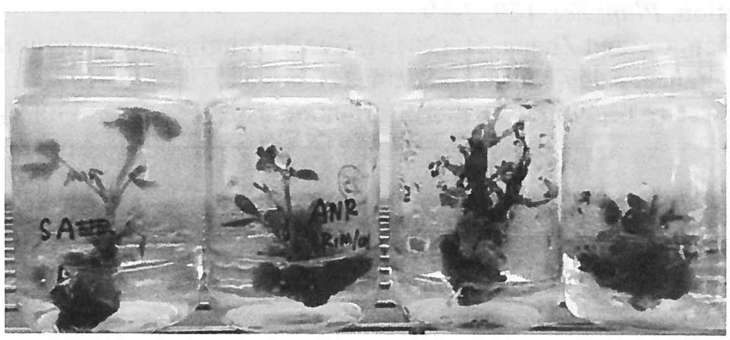

Fig. 6. Putatively transformed peanut plants are growing in media.

Conclusion

Without continuing support from NPB or TPPB, this project will have to come to an end. We will terminate this project at the end of August, 2014. In summary, we have been able to show that it is possible to make peanut significantly more tolerant to salt, drought, or to both salt and drought simultaneously by overexpressing foreign genes such as *AVP1*, *AtNHX1*, and *IPT* (Qin et al., 2011 and 2013; Banjara et al., 2012). We anticipate that as climate change brings dramatic changes in agricultural production in the US, especially in West Texas, there will be a need for creating heat-, drought- and salt tolerant crops including peanut. We are one step ahead in preparing peanut for the unavoidable outcome of climate change.

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