SELECTING GENOTYPES OF VALENCIA PEANUTS FOR SALT TOLERANCE AND EFFICIENT SALINE WATER UTILIZATION

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ABSTRACT

More than one million acres of New Mexico's land area are irrigated annually for crop production. Fresh water scarcity, escalating energy costs for deep well pumping, and increasing population demands for fresh water have restricted the expansion of irrigated agriculture. Problems such as these have caused a decrease in irrigated acreage devoted to low value crops (per unit area), such as wheat and grain sorghum.

Hydrological survey results indicate that New Mexico has an abundance of underground saline water (about 15 billion acre feet). Because of its high content of total dissolved solids, saline water cannot be used for industrial or domestic use, or for traditional irrigated agriculture. Desalinization is expensive and requires a large amount of energy. Agricultural scientists are selecting and breeding crops of economic importance to New Mexico that could produce under culture with high levels of salinity. This project describes such an attempt with Valencia peanuts, a relatively profitable crop under cultivation in the high plains of eastern New Mexico.

Several hundred genotypes of world collections of Valencia peanuts have been collected and screened at levels of salinity that cause severe injury to commercially grown Valencia peanut cultivars. At seedling stage, plants of several accessions were greener, more vigorous and had higher dry weight than the standard cultivars. More research needs to be done in using tissue and cell culture techniques for stress screening purposes and in regenerating the promising callus and cells into agronomically desirable peanut plants while retaining the salt tolerance property.

Keywords: Irrigated agriculture, salinity, salt tolerance*, genotypes, Valencia peanuts*, screening, biotechnology.

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INTRODUCTION

New Mexico possesses approximately 20 billion acre-feet of underground water of which more than three-fourths is classified as saline or brackish (total dissolved solids at least 1,000 ppm). According to the 1980 New Mexico Agricultural Statistics, 1.4 million acres of the state's land area were used for irrigated agriculture. Fresh water scarcity, escalating energy costs for deep well pumping, and increasing population demands for fresh water have restricted the expansion of irrigated agriculture. Problems such as these have caused a decrease in irrigated acreage devoted to low value crops (per unit area), such as wheat and grain sorghum.

In certain areas, saline water is being utilized through improved agrotechnical and drain methods and O'Conner (1980) reported such research on using saline water for crop production in New Mexico. Unfortunately, efforts toward alleviating salinity by reclamation are becoming increasingly cost prohibitive and energy inefficient. Epstein et al. (1980) pointed out the need to develop genetically altered crop plants for culture under saline conditions. This kind of breeding effort, in conjunction with environmental manipulation might make agricultural production in saline and marginal regions economically feasible. Most crop species, however, cannot withstand even moderately high salinity as indicated by detailed assessment studies of Mass and Hoffman (1977), and Francois and Mass (1978, 1985) at the U.S. Salinity Laboratory in Riverside, California.

Valencia peanuts (Arachis hypogaea L. Subsp. fastigiata var. fastigiata) are an important cash crop in New Mexico and worldwide. In New Mexico, Valencia peanuts are grown mainly in the high plains of eastern New Mexico because of an existing acreage and poundage quota allotment. Potentially the peanuts can be grown under irrigation over a wide area of the state, once production quota restrictions are removed in the United States. Valencia peanuts in eastern New Mexico have been irrigated for long periods of time

(50-60 years in many instances) with some well water containing a high concentration of salts (about 3 dS/m). However, salt damage from poor management practices seriously threatens the continued profitable peanut production in eastern New Mexico. Salt damage is the result of a combination of factors including using salty barnyard manure for improving the organic matter content of some newly cultivated fields, little or no leaching due to insufficient natural rainfall, and the ever increasing use of overhead sprinkling systems. In recent years, salt damage to Valencia peanuts has been observed in increasing frequency in different fields or in isolated spots in the same field. Symptoms of salt damaged plants include reduced vigor, poor top growth, severe leaf burns and defoliation. In severe cases, salt damage results in little or no pod production, or death.

New Mexico has a scarcity of fresh water resources but an abundance of saline water. Saline water cannot be used for industrial or domestic consumption, or for traditional irrigated agriculture. Desalinization is expensive and requires a large amount of energy. Selection of peanuts for salt tolerance may bring success in using saline water for irrigating peanuts.

Continuing profitable peanut cultures in New Mexico salt affected areas depend on maintaining satisfactory yield and quality levels under salinity conditions. The conditions bearing on this particular problem are prevalent around the Western United States and the semi-arid and arid regions of the world.

An extensive search of published literature and personal communications with leading peanut scientists throughout the world indicated that the study reported in this publication represented the first attempt to select genotypes of Valencia peanuts for tolerance to high salinity and for utilization of saline water. Considerable genetic variability existed in Valencia peanuts as reported by Hsi (1960, 1978, 1986).

Francois and Mass edited an exhaustive indexed bibliography on "Plant

Responses to Salinity" in 1978 and a supplement in 1985. Epstein and his associates have been working for some time on the saline culture of crops, particularly on barley, wheat, wheatgrass, cotton and tomatoes (1976, 1980). Researchers in Israel developed information on the tolerance of crop plants to salinity based on total yield. Research on the response of peanut yield to saline water was carried out by Shalhavet and his co-workers in Israel (1969). They used only one cultivar, Sholamit (a Virginia type peanut). In that study, the peanut yield curve crossed the range of two salt tolerance groups, sensitive and moderately sensitive, as classified by Mass and Hoffman (1977). This response curve should be verified to find out whether the peanut has such a specific response or perhaps management practices during these experiments lead to the large difference in response. Barnstein (1963) found that osmotic adjustment determined plant growth under saline conditions.

Related works on other crop plants have been attempted by several investigators in the western states. In a few crops, a genetic approach to saline culture of crop plants has shown promise. For example, salt tolerant barley strains from a selection program in California (Epstein et al. 1980) were able to produce satisfactory yield under irrigation with sea water containing 35,000 ppm dissolved solids. Rush and Epstein (1980) hybridized a commercial tomato cultivar with highly salt tolerant wild species of tomato and obtained selections from segregating progenies capable of producing fruits when irrigated with 70% seawater - a level detrimental to ordinary tomato plants. Epstein and his associates also selected a number of spring wheat lines capable of producing grains under irrigation with 50% seawater. The key to breeding success undoubtedly lies in the availability of genetic variability within the germplasm of each crop species and its relatives with regard to salinity tolerance.

The applications of tissue and cell culture methods in plant improvement have been thoroughly reviewed by Tomes (1982) and Vasil (1980). Considerable

progress with the development of tissue culture technologies for peanut have been made only recently. Callus cultures and regeneration of plants from primary explants and young callus has been reported by Bajaj (1981), Mroginski (1981) and Pittman et al. (1983). Peanut plants have been obtained by Pittman et al. (1983) from embryo cultures. Joshi (1968) and Julien (1970) reported worked on peanut cell suspension cultures. Extensive literature review and personal communications revealed no success of plant regeneration from single peanut cells. Embryonic suspension cultures of soybeans have now been achieved and seeds are produced from such regenerated plants. There is apparent need for more tissue culture work to develop regenerable suspension cultures of peanuts. Recent work by Phillips et al. (1983) indicates picloram is a superior auxin for crop plant regeneration and for that of certain wild species of Arachis, including Villosulicarpa and monticola. However, Johnson (1986) obtained prolific regeneration of A. villosulicarpa using only benzyladenine and indoleacedic acid.

Nabors and his group (1976, 1980, 1985 personal visit) used increasing levels of salt as selection pressure for tissue and cell cultures of several crop plants. In 1980, they demonstrated the effective use of plant tissue culture techniques in producing lines of cells and regenerated tobacco plants with increased salt resistance. They have obtained laboratory cultures tolerant to very high salt content. Efforts are being made to regenerate plants from those salt tolerant cultures and to further evaluate their plant responses to salt stress.

The objectives of this study were to:

1. Select genotypes of Valencia peanuts for tolerance to high salt content, and
2. Measure plant response to saline water.

MATERIALS AND METHODS

Valencia Peanut Germplasm

More than 700 hundred world collections of Valencia peanuts were obtained from the U.S.D.A. Southern Regional Plant Introduction Station and used at the Agricultural Science Center at Los Lunas. In addition, three local commercially grown cultivars, New Mexico Valencia A, New Mexico Valencia C, and McRan, plus 12 advanced strains from the Valencia peanut breeding program were also included for the study.

Levels of Salinity. On the basis of a personal visit and communication with the scientists at the U.S. Salinity Laboratory in Riverside, California, the saline solutions were prepared by mixing equal proportions of NaCl and $CaCl_2$ to distilled water and adjusted to three electrical conductivity levels, 3.4 dS/M, 3.7 dS/M and 4.0 dS/M. These levels represented nearly 1,600, 1,800 and 2,000 mg/L Total Dissolved Solids (TDS).

Screening of Valencia Peanut Germplasm (at seedling stage) for salt tolerance. Due to the inability of obtaining satisfactory peanut seedlings from seed soaked in low levels of saline solution and also due to the erratic emergence of seedlings from the plastic tubes filled with sterilized perlite, seeds of Valencia peanut germplasm to be screened were first surface disinfected with seed treatment compound (Botec) and then placed in water agar plates. The young seedlings from individual accessions were then transplanted into the 6" plastic tubes, which were placed on metal racks. They were then allowed to grow in aerated distilled water in big 16" x 22" plastic tubs. The tubs were differentiated by colors with brown indicating the high level of salinity, yellow the medium level and white the low level of salinity. As soon as the roots emerged from the plastic tubes into the tub water, saline water of different levels of electrical conductivity replaced the distilled water. Hoagland solution (1938) was added to the saline water after the seedlings approached three weeks in growth, and electrical conductivity was adjusted by means of a salinity sensor and

metering device. Each plastic tub holds 64 plant tubes and each group of materials screened were replicated three times.

Plant Response to Saline Water Under Hydroponic Growing Conditions. Light was supplied by GTE Sylvania Grow Light (Sun Stix, 118V 60hz ZOW) for 14-hour photoperiod at room temperatures in the laboratory. Water was continuously aerated in the tubs by means of individual aerated pumps for tropical fish tanks. Notes were taken of color and vigor of seedlings and plant height. Individual series of experiments were terminated after 12 weeks. Then another series of experiments using a different group of germplasm started. In all experiments, New Mexico Valencia A, New Mexico Valencia C and McRan were used as checks.

Tissue and Cell Culture Studies for Possible Use in Screening Germplasm for Salt Tolerance. Nabors and his group (1975, 1976, 1980) have been using callus and cell lines of several crop plants for producing mutant plants conducive to salt tolerance. Mroginski et al. (1981) and Pittman et al. (1983) have reported success in regenerating peanut plantlet by *in vitro* culture of immature peanut leaves. It appears that tissue and cell culture technology will be a useful tool in screening peanuts for salt tolerance. Upon the advice of Dr. Phillip Gregory and his assistants at New Mexico State University, attempts were made in initiating tissue and cell cultures studies with Valencia peanuts and related species at the Agricultural Science Center at Los Lunas.

Seeds of NM Valencia A, NM Valencia C and McRan, with testa (seed coat or skin) removed, were germinated aseptically on sterile water agar. Immature leaflets from 8- to 12-day-old seedlings were used as explants and cultured on either solid basic media of Murashige and Skoog, or Linsmaier and Skoog as modified by Phillips (1983) and Nabors (1980). All media were adjusted to pH 5.8 with 1N NaOH or 1NHCl prior to steam sterilization. Sterile polystyrene petridishes containing solidified nutrient media were plated with four explants under a sterile transfer hood and then sealed with

Parafilm. Cultures were incubated at 25°C under constant 16-hour light. Good development of callus was usually observed in three to five weeks. Portions of callus were placed in 150 ml. flasks containing liquid media which were then placed on a gyrotory shaker. The mechanical motion of the shaker disrupts the callus into small clumps of cells and single cells (a cell suspension). At weekly intervals, dense cell suspensions were subcultured into fresh liquid media.

Three levels of salt content were used for selecting mutants in the cell suspension that might be tolerant to high salinity. A combination of 85% NaCl and 15% $CaSO_4$ was added to the liquid medium. The high salinity solution contains 9,000 mg/L TDS and the low containing 5,000 mg/L TDS.

RESULTS AND DISCUSSION

Each series of screening tests consisted of 61 strains or accessions and three standard checks and took about 12 weeks. Seven series of tests were conducted. More than 400 world collections and 12 advanced strains were screened for salt tolerance. The extremely salt susceptible strains were wilted, severely stunted, or chlorotic at the end of four weeks in hydroponics containing high salt content. The two higher levels of salinity, 4.0 dS/M, and 3.7 dS/M, were more useful in critically evaluating the salt tolerance of Valencia peanut germplasm in this study.

Rather than a detail listing of all the test data of several hundred peanut collections in this report, only those plant introductions that were most tolerant to salinity and the three standard cultivars are described in table 1. The most salt tolerant collection among the materials tested was P.I. 268508 from Rhodesia (Zimbabwe). It showed green and vigorous growth and was taller than the standard varieties under salt stress conditions. There appeared to be a good association between plant vigor, height and dry weight of the whole plant.

Due to the time and space limitation, only a small number of the whole plants could be tested from each accession of the world collection. Furthermore, the salt tolerant seedlings were not grown to maturity to produce seed increase. Because the genetic purity of each collection was not known, repeated screening tests for salinity will be necessary to ascertain the overall salt tolerance quality of each accession before actually using it as a possible genetic source for gene transfer in a breeding program. Because considerable time was spent in developing a satisfactory screening procedure for this project and also because of severe time and budgetary constraint, only two-thirds of the Valencia peanut world collections were evaluated and consequently repeated screening tests of the same materials were not possible in this study.

Table 1. Description of Several Salt Tolerant Valencia Peanut Accessions and Standard Check Cultivars, Agricultural Science Center at Los Lunas, 1984-85.

Entry	Source	Testa Color	Dry Weight** 8-week old Peanut Seedlings 3.7 dS/M	4.0 dS/M	Avg.	Plant Height**
			g	g	g	in.
P.I. 268508*	RHOD	Red	9.5	4.4	6.9	14
P.I. 264189	AUSTL	Red	5.8	1.6	3.7	14
P.I. 259769	NYASA	Red	4.2	3.0	3.6	10
P.I. 259598	VENEZ	Red	2.2	3.0	2.6	12
P.I. 259735	ARGN	Red	2.3	2.9	2.6	11
P.I. 259732	ARGN	Red	2.0	3.0	2.5	10
N.M. Valencia C	USA	Red	2.3	1.7	1.9	10
N.M. Valencia A	USA	Red	2.2	1.4	2.0	9
McRan	USA	Red	2.0	1.1	1.6	8
Tests Avg. (n=144)			2.0	1.3	1.6	7

* Plant Introduction Number assigned by the Plant Induction Stations of the U.S. Department of Agriculture
**Average of 3 replications for accessions and 6 replications for checks

Because of the limitations of time, space and budget in most research programs, tissue culture technology offers a useful and economically feasible selection tool to the plant breeder. Because in genetic terms each cell represents a potential plant, thousands of potential plants can be maintained in a single vial and billions in a small room. According to Nabors and others, desirable mutations (for specific traits) occur very infrequently (perhaps in one of every million plants), the expense involved in field selection alone not only is considerable but also is uncertain of any degree of success. In a case of selection for a trait like salt tolerance, the entire process, for millions of cells, can be performed in a few flasks in which uniform application of selection stress or pressure can be applied.

Our preliminary exploratory works with peanut tissue and cell cultures, however, were not consistent enough to offer this technology as a promising tool for salt stress screening purposes. We have been able to regenerate only a few plantlets from many calli of locally grown Valencia cultivars. We have not been able to regenerate plants from cell cultures. As experienced by Pittman et al. (1983, 1984), Sellars et al. (1983), was more successful in plant regenerations from a wild peanut species, Arachis villosulicarpa Hoehne than from cultivated peanuts.

Preliminary works with cell suspensions growing in saline solutions were also inconclusive. Reduced growth and death of cell cultures were observed at the high salinity level. No salt tolerant cells were ever detected in our limited study.

Nabors and his co-workers at Colorado State University have been conducting extensive research on the development of stress resistant plants from tissue and cell culture. The steps outlined by Nabors to produce salt tolerant plants using tissue culture methods are that:

1. A large number of cells sufficient to contain the desired mutant trait must be produced;
2. A selection procedure designed to a culture of useful mutant

cells must be utilized;

3. Plants must be regenerated from salt-stress resistant culture and from non-resistant cultures;
4. Plants obtained from stress-resistant cultures must be shown to retain the salt resistance or tolerance to pass it on in a predictable fashion to subsequent generations; and
5. Trials must demonstrate that progeny of regenerated salt tolerant plants are useful or agronomically desirable under stress conditions encountered in the field.

The use of salt tolerant materials from whole plant screening might increase the chances of obtaining salt resistant or tolerant mutants in steps 1 and 2 mentioned above.

Because plant regeneration of wild peanut is more consistent than the cultivated peanuts and because the haploid or anther culture has been used successfully in tissue culture work of several crop plants, developing methods for protoplast fusion will make available to peanut tissue culture workers and breeders another useful technique for selecting and developing new salt tolerant plant types in addition to sexual crosses and conventional plant breeding method.

Use of tissue and cell culture technique for developing stress resistant plants are being intensely investigated in well equipped laboratories located at Colorado State University and New Mexico State University. Their findings and guidance will be helpful toward using this kind of biotechnology in developing salt tolerant Valencia peanuts in the future.

SUMMARY AND CONCLUSION

Results from salt screening tests conducted under hydroponic growing conditions in this study have shown that salt tolerance existed in certain Valencia accessions of the world collections.

Exploratory works with tissue and cell culture indicate that this technology does not appear feasible for use as a screening tool for salt stress at this time. With more success with plant regeneration from cell and tissue culture and with more definitive findings from well equipped laboratories such as those located at Colorado State University and New Mexico State University, *in vitro* screening procedure utilizing explants from salt tolerant seedlings may offer a powerful tool in germplasm screening for tolerance to salt stress in the future.

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