



MICROPROPAGATION OF POTATO VARIETIES AND THEIR IN VITRO CHARACTERIZATION AGAINST SALT STRESS

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ABSTRACT

Two potato varieties namely *Kuroda* and *Paramount* were collected from the Agriculture Research Institute Tarnab Peshawar, KPK, Pakistan, for their micropropagation and then characterizing them in vitro against the salt stress. This process was performed at the Plant Tissue Culture Laboratory in Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture Peshawar, Pakistan. The potato varieties were first allowed to sprout under suitable conditions. Then the buds obtained from sprouted potatoes were used as their explants for micropropagation. These explants were cultured on Murashige and Skooge (MS) medium in test tubes. Some of them were used as control with no salt. While in the remaining test tubes three different salt concentrations i.e. 25mM, 50mM and 75mM were used. After few days various parameters of the cultured buds such as rooting and shooting response, the number of roots formed and the difference in salt stress responses of the two potato varieties was determined. It was deduced that there is no clear relation between the growth parameters and the salt concentration. Still it was seen that both of the potato varieties are highly adaptive.

Key Words: micropropagation, in vitro, Kuroda, Paramount, salt

INTRODUCTION

Nowadays, potato ranks fourth among the staple food of mankind after wheat, rice and maize (Diretto et al., 2007). It is already an integral part of the global food system. Among the main non grain foods it is on the first position in the world. So far its record production has been calculated to be 325 million tons (Joshi and Gurung, 2009). The use of potato is increasing day by day. It's easy to cultivate, handle and cook. It's nutritious and tasty. Used and liked by all age groups.

Pakistan's economy is mainly based on agriculture and produce about 24% of the total. It provides up to 50% of the jobs (Hamid et al., 1990). At the time of independence (in 1947) the area under potato crop was 3 thousand hectares, which increased to 40.5 thousand hectares during 1990 - 2000, with the total production of about 1.8 million metric tons (mmt), out of which 0.28 mmt is used as seed and remaining is consumed (MINFAL, 2000). With the population of 150 million, this accounts to 11 kg per capita per annum. During the last ten years, the yield per hectare has increased by 60% due to improved seed and better crop management practices. Tubers are the most common source of potato reproduction. Approximately 15% of the total area under potato cultivation around the world is used for the production of seed tubers

(FAO, 2000; Kanwal et al., 2006). A large number of potato varieties are grown in Pakistan. They are broadly divided in red skinned and white skinned varieties. The Red skinned varieties include *Desiree*, *Cardinal*, *Raja*, *Synphonia*, *Kuroda*, *Barna*, etc. the White skinned varieties include *Sante*, *Santana*, *Diamont*, etc. (Kokab and Smith, 1989).

Salinity is one of the major abiotic stresses that adversely affect crop productivity and quality. Management of water and land can be successful in reclamation of salt affected soil (Brestein, 1975), but the most economical and effective means is to plant crops that can establish and be productive on such soils. Therefore, impact of salinity on plant can also be managed through biological manipulating the plant (Rains, 1981). Identification of plant genotypes capable of increased tolerance to salt and incorporation of these desirable traits into economically useful crop plants may reduce the effect of salinity on productivity.

Biotechnology is the art of utilizing living organisms or their products for the benefit of mankind or other living organisms. It is a combination of Genetic Engineering and Tissue Culture. In Genetic Engineering desired genes selected and taken from one organism may be transferred to another organism whether it is of the same species or of another species. Genes may be transferred to an organism of a different kingdom. The intraspecific barrier of gene transfer during the mating process of organisms is overcome. It is suggested that in the case of salinity problem, biotechnology may play an important role. Various varieties of plants are screened for stress tolerance. Stress tolerant plants are selected for further study.

In this research an attempt was made to check the stress tolerance level of various potato varieties by using *in vitro* technology. *In vitro* technology has a benefit over the field by the fact that it provides a controlled environment and requires less time. The nutrients component and various factors such as temperature, pH, etc. are kept fixed. This *in vitro* screening or characterization of potato varieties against NaCl stress is an initial step in crop improvement to tolerate soil and water salinity.

MATERIALS AND METHODS

The present research experiments were carried out at the Plant Tissue Culture Laboratory, Institute of Biotechnology and Genetic Engineering (IBGE), Khyber Pakhtun Khwa (KPK) Agricultural University Peshawar (AUP), during September and October 2012. The experiment was repeated three times.

a) Potato sprouting

Two varieties of potato namely *Kuroda* and *Paramount* were collected from the Potato Botanist Syed Azhar at Agriculture Research Institute (ARI), Tarnab on 20th September, 2012. These potatoes along with their respective paper labels were put into two separate plastic bags. Then these potatoes were transferred to two separate labeled trays. They were then kept at warm place in shadow in open air for three days, so that the potatoes may sprout. After three days, on 24th of September, the potatoes were checked to see whether they have sprouted or not. The result was positive i.e. the potatoes were sprouted. At this, the sprouts or the buds were small enough and were not able to be used as the explants. So they were allowed to grow further for about a week or more. On October, 10, it was found that the buds have grown sufficiently enough and were able to be used as the explants (see figure 1).

b) Collection and Sterilization of the Apparatus

Various apparatus needed in the experiment were collected. These apparatus included test tubes, beakers, conical flasks, magnetic stirrers, forceps, scalpels, etc. The glass apparatus were first carefully washed with detergents, inverted for drying and then sealed with aluminum foil (see figure 2). All these glass vessels were then autoclaved (as shown in figure 3). The forceps and scalpels were autoclaved too.

c) Labeling of the test tubes

All the 40 test tubes of the first experiment were divided into two equal groups with 20 test tubes each. All the test tubes of one group were labeled as “K” for the *Kuroda* and those of the second group as “P” for the *Paramount* variety. These 20 test tubes of each variety were divided into four groups with 5 test tubes each. They were labeled as “C, 25, 50 and 75”, denoting the milli molar (mM) concentration of the salt present in the medium to be poured in the test tubes (as is mentioned in the figure 4).

In the same way all the 24 test tubes used in the second experiment were also divided into two groups, each having 12 test tubes. All the test tubes of one group were labeled as “K” for the *Kuroda* and those of the second group as “P” for the *Paramount* variety. These 12 test tubes of each variety were divided into four groups having 3 test tubes each and they were labeled as “C, 25, 50 and 75”, denoting the concentration in milli moles (mM) of the salt present in the medium.

d) Full MS Medium Preparation

Full MS (Murashige and Skooge) medium was used as the culture medium. The detailed composition of which is given at the last of this report. Various stock solutions were already present in the laboratory. Based on these stocks our required volume of the medium for both experiments was obtained.

- A. In the first experiment 500ml of the MS medium was prepared for 40 test tubes. For this small amount of distilled water was added to a one liter beaker. See figure 5. Then according to the calculations, we added 50ml of macronutrients, 5ml of micronutrients, 50ml of the vitamins and 50ml of the iron source stock solutions respectively. Then we added 7.5g of sugar. The volume of the solution was reached to 500ml by adding distilled water to it in a measuring cylinder. The solution was again poured back to the beaker. 100ml of this solution was transferred to four conical flasks each. These flasks were labeled as control (c), 25mM, 50mM and 75mM denoting the amount of NaCl present in it. In control no NaCl was added. In 25mM, 50mM and 75mM flasks 1g, 2g and 3g of NaCl was added respectively to get the required mentioned concentration. Using a pH meter the pH of the medium was adjusted round about 5.8 (see figure 6). After the pH adjustment 1g of agar was added to each flask. Then this solution was heated in an oven. Soon after the removal of these solutions from the oven, such that they were still hot, they were transferred to their respective labeled test tubes. These test tubes were then sealed with aluminum foil and put in racks. Finally all of them were autoclaved.
- B. In the second experiment we prepared 300ml of the Full MS media. For this we took about 100ml of distilled water in a beaker. From the stock solutions added 30ml of macronutrients, 3ml of micronutrients, 30ml of vitamins, 12ml Tang and 30ml of the iron source solutions from their respective stock solutions. To this solution we added 0.03g inositol and 4.5g sugar. This solution was transferred to a measuring cylinder. The volume was raised to 300ml by adding distilled water to it. This solution was mixed with magnetic stirrer. Then it was equally distributed in four conical flasks each containing 75ml of the medium. These conical flasks were labeled as C, 25mM, 50mM and 75mM. To flask “C” no salt was added, to “25mM, 50mM and 75mM” flasks we added 1.1g, 2.2g and 3.3g of NaCl to get the required amount of it in the media. Using pH meter the pH was adjusted round about 5.8. After the pH adjustment 0.6g of agar was added to each flask. Then this solution was heated in an oven. Then this hot

solution was transferred to their respective labeled test tubes (as shown in figure 7). These were then sealed with aluminum foil and autoclaved.

e) 20% v/v sodium hypo-chloride

20% v/v sodium hypo-chloride solution was prepared for the sterilization of the explants. 20ml of sodium hypo-chloride solution was taken from its stock solution and was poured in a 100 ml graduated cylinder. Distilled water was added to it till the solution volume became 100 ml. the solution was poured in a flask and mixed with a magnetic stirrer.

f) 0.1 % mercuric chloride

To prepare 0.1% mercuric chloride solution we took 100ml of distilled water in a graduated cylinder and added 0.1g of mercuric chloride to it. Then we poured it in a flask and mixed it with a magnetic stirrer.

g) Cutting and sterilization of the explants

On 12th of October, the potatoes were taken. Their buds have grown well to be used as the explants. Two small conical flasks were taken and washed. These flasks were labeled as “K” and “P”. The buds were cut with scalpel into one, two or three parts depending upon the size of the bud to be cut as is shown in figure 8. The buds from *Kuroda* were put in the flask labeled as “K” and those of *Paramount* were put in the one labeled “P”. After cutting these buds were first washed with tap water, then with distilled water and at last they were treated with 20% v/v solution of sodium hypo-chloride for about 8min in the flask. The bleach was poured off the flask and the buds were washed with distilled water two to three times to remove the traces of the bleach.

In the second experiment all the procedure of cutting and sterilization of the explants was the same with the addition that the buds were treated with 0.01% w/v mercuric chloride solution for 3 min, after the treatment with sodium hypo-chloride. After this the buds were washed with distilled water to remove the traces of the bleaches.

h) Transfer of explants to the test tubes

First of all, the Laminar Flow Chamber (LFC) was opened and sprayed with bleach. Then it was dried with cloth and tissue paper. Then all the materials which were used in the transfer of the explants were transferred to it. These materials included racks containing the autoclaved test tubes and forceps, bleach solution, flasks containing the sterilized explants, match box and spirit lamp. The spirit lamp was lit. The forceps was sterilized on fire. Buds were first taken with the help of the forceps from the flask labeled “P” and transferred to the test tubes labeled “P” one by one. Then in same way buds from the flask labeled “K” was transferred to the test tubes labeled “K”. all these test tubes were covered with their respective aluminum caps as soon as the bud was transferred to it and the mouth of the test tube was heated with flame(see figure 9).The test tubes were put in their racks.

i) Explants germination

For the germination of the explants in the test tubes they were transferred to the Growth Chamber in order to provide controlled environment. Rooting and shooting was checked at various intervals to collect data(see figure 10 & 11).

List of Figures “A”



Figure 6: Potatoes Sprouting



Figure 3: Test tubes washing



Figure 2: Autoclaving glass wares



Figure 1: Labeling



Figure 4: Water poured in beaker



Figure 5: Adjusting pH of the media

List of Figures “B”



Figure 12: Pouring hot media in test tubes



Figure 11: Cutting explants



Figure 10: Transferring explants to test tubes



Figure 7: Explants germinating



Figure 8: Explant showing clear rooting



Figure 9: Explant grown to whole plant

RESULTS AND DISCUSSIONS

On 7th of November 2012, data was taken as the explants have grown to complete plants (as shown in figure 12). Various parameters of both varieties including number of roots and shoots and length of the longest root and shoot observed, at various levels of NaCl concentration applied to the medium was noted. Using Microsoft Office Excel this crude data was plotted in graphical form (as shown in figure 13, 14,15 and 16), by first taking the average value for the three and then plotting it against the respective concentration.

When the concentration of NaCl was raised from 0 to 25 mM the average root number of varieties of potato was decreased (see figure 13) and the average root length of *Kuroda* was decreased while that of *Paramount* was highly increased (as shown in figure 14).

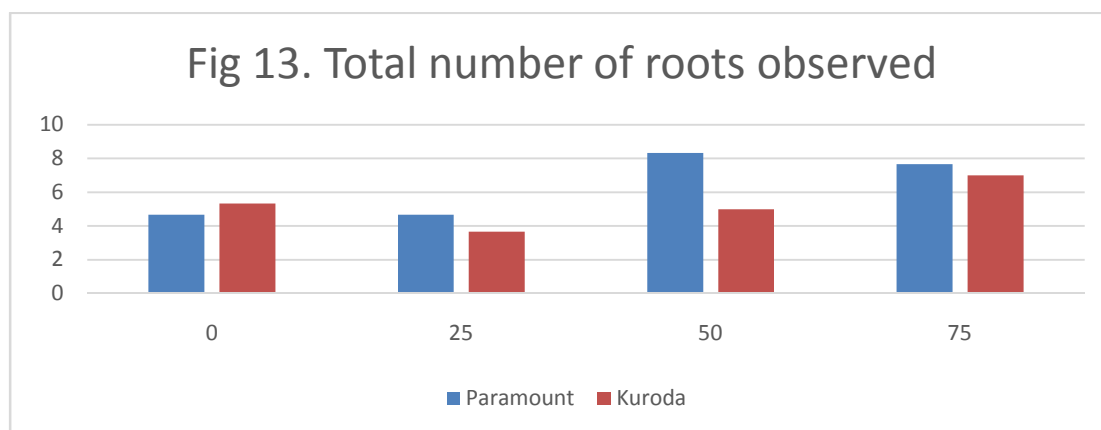
When the concentration of NaCl was raised from 25 to 50 mM the average root number of the average root number of both varieties was increased (see figure 13) while the average root length of *Kuroda* was slightly decreased while that of *Paramount* was slightly increased (see figure 14).

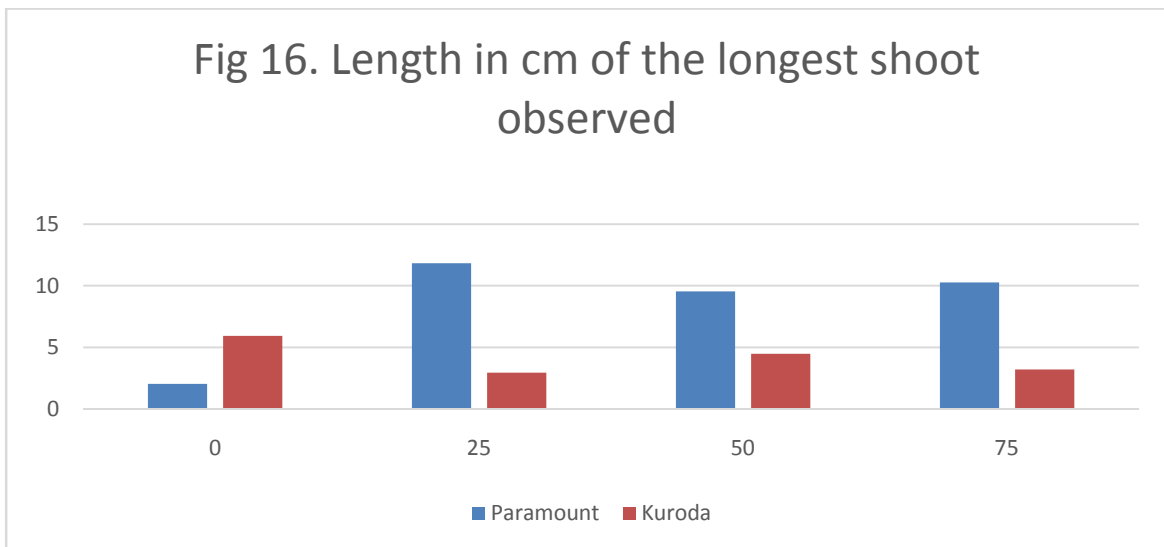
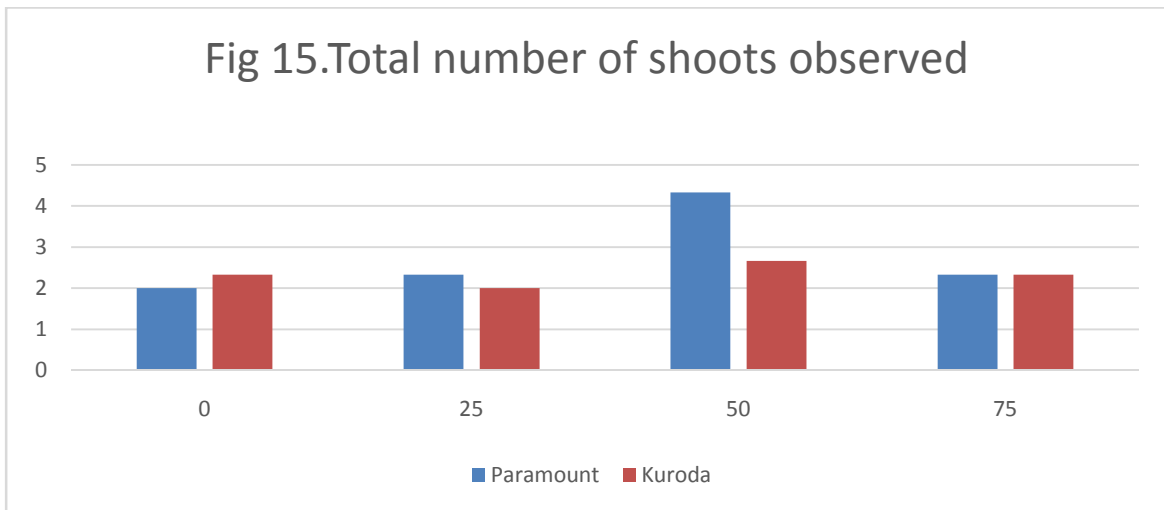
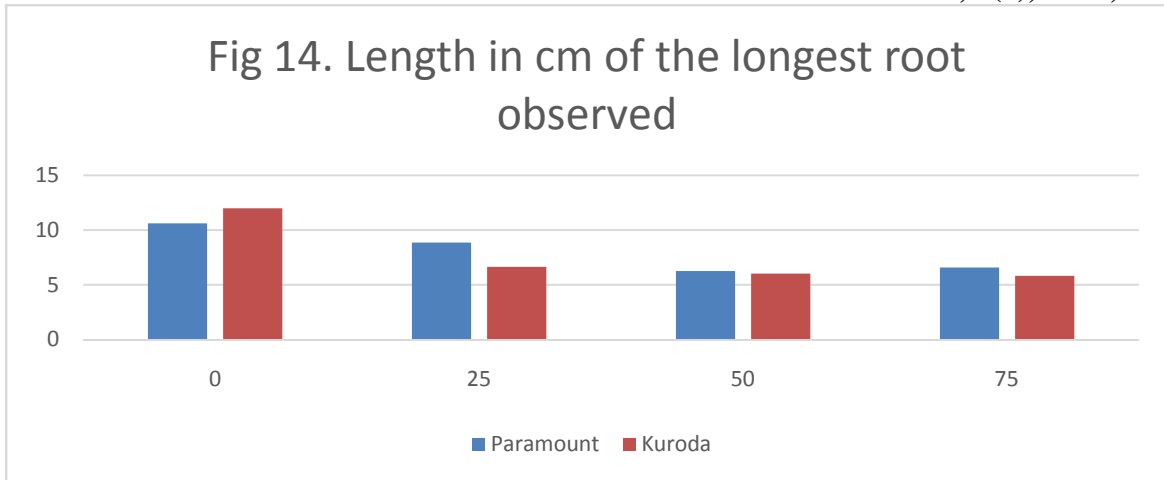
When the concentration of NaCl was raised from 50 to 75 mM the average root number of *Paramount* was increased while that of *Kuroda* was decreased (see figure 13) and the average root length of *Paramount* was decreased and that of *Kuroda* was increased(see figure 14).

Total number of shoots (fig. 15) showed that there were significant differences in the interaction of salt stresses and potato varieties. Maximum number of shoots was recorded for both cultivars at 50mM concentration of salt stress but the response of *Paramount* was comparatively higher than *Kuroda* at this level of salt concentration.

Length of the shoot of both varieties at various levels of salt concentration (fig. 16) showed irrelevant trend. In the control treatment *Kuroda* gives maximum shoot length than the paramount as well as its response at respective other levels of salt concentrations. The response of shoot length of *Paramount* varieties on the salt stress gives better shoot length than the control. Maximum value was of shoot length of paramount was recorded for 25mM concentration.

From the above results it is concluded that there is no fixed relation (direct or indirect) between the growth parameters and the salt concentration. This sort of conclusion was also derived by Cano et al. (1998) who tested salt tolerance in tomato varieties *in vitro*. He found that shooting response to various levels of salt concentrations by the apices of the two tomato varieties was different. This difference in the shooting response was not clearly understood. Whereas Troncoso et al. (1999) carried out research experiment on grapevine *in vitro* and found that there is a decrease in rooting with increase in salt concentration.





RECOMMENDATIONS

- Explants of the same size should be used.

- The range of salt concentration should be increased.
- Data should be taken at fixed regular intervals.
- The sterilization of the explants is quite sensitive so it is better to use germ free explants. For this, first of all, the varieties should be grown on controlled medium and then explants should be taken from these germ free cultures.
- Need further detailed *in vitro* research.

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