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Full Length Research Paper

In vitro conservation of exotic potato genotypes through different incubated temperatures, aerophilic and micro-aerophilic conditions

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The present study was carried out to study the *in vitro* conservation of potato genotypes at different temperatures and aerophilic and micro aerophilic conditions. A total of 31 genotypes were conserved at different incubated temperature ranging from 10 to 25°C. At lower temperature (10°C) plant growth was slowest as compared to plants incubated at high temperature (25°C). The results revealed that aerophilic condition was optimum for the growth of all potato genotypes. Data were collected on plant height, number of roots and number of nodes. Maximum plant height, highest number of roots and number of nodes were observed in all genotypes grown at 25°C. *In vitro* microareophillic condition of the plant growth was very slow but conservation was maximum. It was concluded from the present investigation that low temperature and micro-aerophilic condition is best for in vitro conservation of International Potato Center (CIP) germplasm which can increase the period between sub culturing.

Key words: *In vitro* conservation, genotype, sub-culturing, aerophilic.

INTRODUCTION

Potato (*Solanumtuberosum* L.) is the most important food crop throughout the world. In financial terms its ranked 4th in the world after wheat, rice and maize. In the world Pakistan is the 7th largest potato producing country (Afrasiab and Iqbal, 2010). Potato is exceedingly heterogenic plant and their germplasm cultivars are therefore needed to be kept up through vegetative propagation as clones, to monitor their hereditary trustworthiness. There are numerous reports on potato micro-propagation and protection that could be possible through in vitro and in situ process (Yousef et al., 2001; Badoni and Chauhan, 2009; Rahman et al., 2010). On-field conservation of potato germplasm through clonal propagation required a lot of time, space and labor. This additionally opens the plants to infections and bugs, and dangers of misfortune because ofabiotic anxieties and

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characteristic cataclysms. Along these lines, all through the world potato gene banks like to preserve parentalclones as in vitro producing micro-plants under ailment free tissue society conditions (Roca et al., 1989).Conservation of plant genetic resources is essential for food security and agro-biodiversity. Genetic diversity provides options to develop through selection and breeding of new and more productive crops, resistant to biological and environmental stresses (Nisar et al., 2011; Rao, 2004). For more food, it will be necessary to make better use of a broader range of genetic diversity across the globe. Many plant species are now in danger of becoming extinct (Panis and Lambardi, 2005). More than fifteen million hectares of tropical forests are vanished each year (Rao, 2004). Their preservation is essential for plant breeding programs. Biodiversity provides a source of compounds to the medical, food and crop protection industries (Panis and Lambardi, 2005). Genetically uniform modern varieties are being replaced with highly diverse local cultivars and landraces of traditional agro-ecosystems. Deforestation, urbanization, pollution, habitat destruction, fragmentation and degradation, spread of invasive alien species, climate change, changing life styles, globalization, market economies, over-grazing and changes in land-use pattern are contributing indirectly to the loss of diversity (Pitman and Jorgensen, 2002). These reductions are a threat for food security in the long term. Gene banks were established in many countries for conservation of plants (Rao, 2004). Advances in biotechnology, especially in the area of in vitro culture techniques and molecular biology provide some important tools for improved conservation and management of plant genetic resources (RamanathaRao and Riley, 1994; Withers, 1995). Conservation of plant genetic resources can be carried out either in the natural habitats (*in situ*) or outside (*ex situ*). *Ex situ* conservation is generally used to safeguard populations, in danger of destruction, replacement or deterioration. An approach to ex situ conservation includes methods like seed storage in seed banks, field gene banks, botanical gardens, DNA and pollen storage (Rao, 2004). Among these, seed storage is the most convenient method of long-term conservation for plant genetic resources.

In vitro conservation of genetic resources has got importance in recent years. Since, last 40 years, CIP has been contributed to developing tissue culture techniques for conserving potato germplasm (Withers et al., 1997).It is the most prominent and efficient way for distributing clonal materials. It ensures the availability of planting material any time and made possible the eradication of virus through meristem culture. Furthermore, in vitro conservation is less expensive as compared to preservation process (Maltaris et al., 2007).

Potato needsub-culturing after every 4 to 6 weeks, to extend the time of sub-culturing, growth retardants in blend with a less energy source, low temperatures and minimum light intensity may be utilized. Hence this part of biodiversity protection must be guaranteed through germplasm accumulations in gene banks where the local hereditary material must be secured and kept up for further utilize. Apropertechnique of potato germplasm storage is the material conservation in slow growth conditions (Sarkar and Naik, 1999). The principle of slow growth storage allows a safe use of in vitro culture without the disadvantages of frequent sub cultivation. The cultures can be observed while they grow and can be returned to normal multiplication subculture (Withers, 1991) and particularly useful for local varieties of potato (Kotkas, 2004; Ciobanuet al.,2011).

Present investigation was therefore, carried out to study the influence of temperature on in vitro conservation of 31 exotic genotypes and to find out the best temperature ranges for multiplication and conservation of potato germplasm. It is aimed that establishing the proper conditions for slow growth in potato will aid in preserving their germplasm for the purpose of later reintroduction and sustainable use.

MATERIALS AND METHODS

Plant materials

A total of 31CIP potato (*Solanum tuberosum L.*) variety was used for in vitro conservation through different incubating temperature 10, 16 and 25°C. For that, explants (1 to 2cm) were inoculated in MS media and incubated at different temperature 25, 16 and 10°C. Enhanced growth rate was observed in plants incubated at 25°C whereas, the slow growth rates were observed in plants incubated at 10°C.

In vitro conservation

To induce a shoot from explants and to cultivate cell in suspension various kind of media have been designed. For the sake of convenience, macro and micro nutrients necessary for plants growth were formerly combined in a definite proportion to form Murashige &Skoog MS (1962) media. One of the commonly used media for tissue culture was that developed by Murashige &Skoog for tobacco tissue culture. Both the over concentrated and poor concentrated media never show satisfactory result. For in vitro conservation,simple media was used without supplement of any plant growth regulators (PGRs) and agar is also added to solidified the media which provide support into the new explant. The previously multiplied explants were used as plant materials.

*In vitro*multiplication was carried out by culturing nodal segment of 31 CIP different genotypes. All the equipments (Forceps, Scalpels, Petri plates) were surface sterilized in an autoclave at 121°C temperature and 15 PSI Pressure for 1h. Under aseptic condition plantlets of the CIP genotypes were taken out in a sterile plate, with the help of sterile forceps. With the help of sterile scalpel, the rootsand leaves of these plantlets were removed and finally shoot part was cut into smallsegment, each segment having at least one node. Maintaining the proper polarity of the cut segment, and inoculated in the culture medium in test tubes (size 25×190 mm, containing 10ml of solidified media). After inoculation, explants cultures were incubated at three different temperatures 10, 16 and 25°C under the light of white fluorescent tubes for 3 weeks. And for the study of aerophilic (tubes covered with plugs) and micro aerophilic (tubes covered with tight Caps) study the explants culture were incubated at the same temperature, that is, 25˚C.

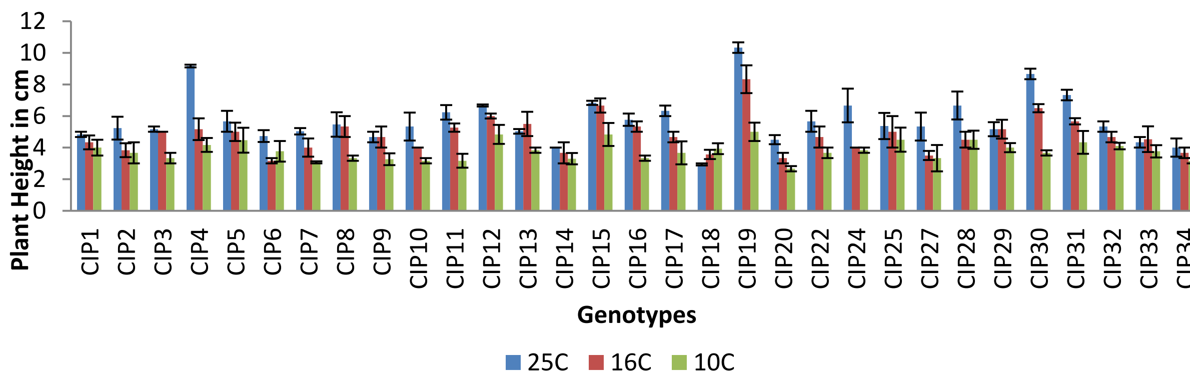

Figure 1. Plant height of 34-CIP potato germplasms grown at different temperature levels.

Picture 1. Plant height and number of nodes of 34-CIP potato germplasms at different temperature ranges.

Statistical analysis

All experiments were established in a completely randomized design. Experiments showing responsive treatments were repeated once. The data were recorded the length of plants and the number of nodes and root per plants with help of ruler and then plotted on the Excel sheet for measuring the mean value andstandard error value. The data were analyzed by descriptive statistics and both the mean andstandard values were used for graphs designing.

RESULTS AND DISCUSSION

In the present study influence of temperature on in vitro conservation of 31 exotic genotypes were examined and monitored for one month. Overall plant height and health were examined during experimentation period.

Plant height

The plant height was recorded visually with the aid of feet meter scale after four weeks. The results revealed that maximum plant height occurred in plants grown at 25°C. Maximum plant height was recorded in CIP 04 and CIP 19 genotypes. In contrast at 10°C, lowest plant height and growth was examined (Figure1andPicture1). Ourresult was also confirmed by Arrigoni-Blank et al. (2014) who reported that other than genotype, temperature effects on shoot height and shoot viability of sweet potato. Similar observation was reported by Boese and Huner (1990) that chlorophyll and carotenoid contents were twofold higher in 16°C than in 5°C leaves on a dry weight basis. It was also shown that the plant grown in lower temperature produced more thick leaves as compared to higher temperature, and is due to 1-4 fold increase in the mean length of palisade and spongy mesophll cell. Gopal et al. (2003) reported that slow-growth *in vitro* conservation of potato germplasm occur by decreasing propagated temperature. Similar report by Ranjbar and Khan (2012) showed that difference in plant

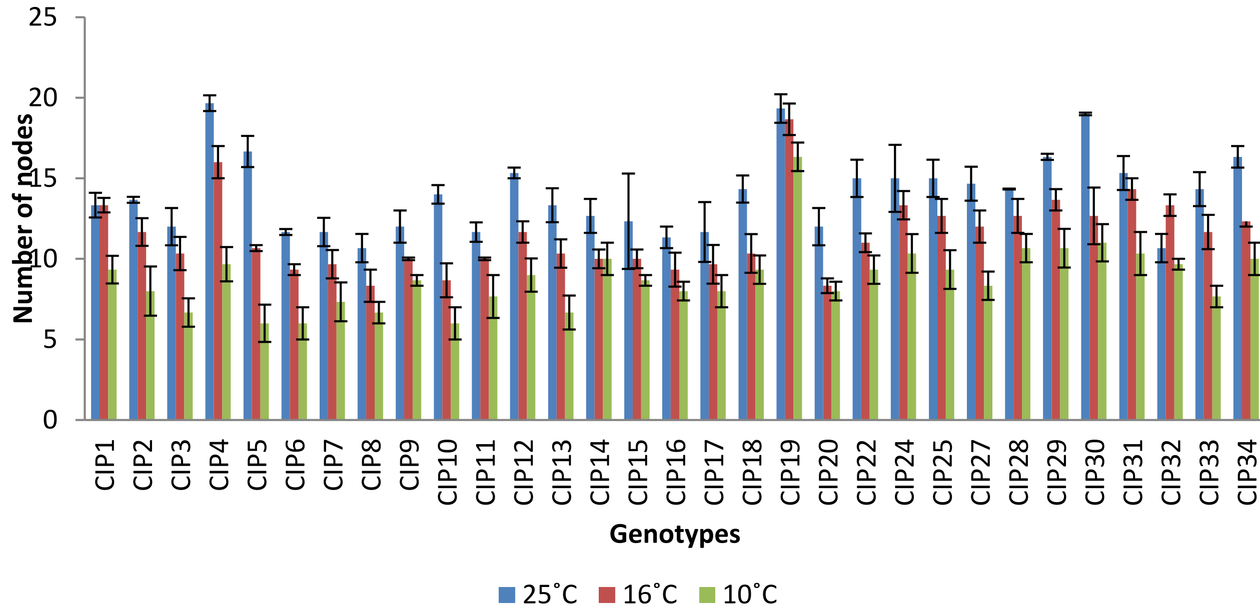

Figure 2. Effects of incubated temperatures on number of nodes of 34-CIP potato germplasms grown at different temperature ranges.

heights in term of length and number of internodes may be attributed to genetic difference in varieties.

Number of roots

The number of roots per plant was counted by visual observation. The results revealed that the plants produced maximum number of roots that were incubated at 25°C as compared to plants incubated at 10 and 16°C. It was concluded that lower temperature can suppress the plant growth to some extent without causing harmful effects.Badoni and Chauhan (2009) reported that highestnumber of roots was produced in plants cultivated at 25°Ctemperature.

Number of nodes

Results showed that the highest number of nodes wasproduced in genotypes incubated at 25ºC. While the lowest numbers of nodes were revealed in genotypes cultivated at 10ºC. It was also revealed that increase in temperature cause increase in plant height as well as in the number of nodes (Figure 2). Similarly, Ciobanu and Constantinovici (2012) also suggested that forconservation of potato, low temperature (6-12°C) wasfavorable as it reduced the ascertained number of nodes.

Effect of aerophilic and micro-aerophilic conditions on growth

The results showed that ventilation was the basic need for growth and development of plant in vitro incubated (condition) environment (Figure3 andPicture2). The CIP genotypes were cultivated in tube covered with plug (aerophilic condition), showed best growth rate and the plants were very healthy as compared to plants grown in caped test tubes (microaerophilic condition). The plug covered plant showed maximum (43%) plant height as compared to cap plant. It was also observed that plant grown in tube covered with plug produced large number of roots then cap covered growing plants. Similar report by Mohamed and Alsadon (2009) showed that using ventilated vessels with low sucrose concentration under ambient CO_2 concentration of the growth room could successfully induce photomixotrophic culture resulting in healthy plantlets. Higher leaf dry weight and anatomically well-developed leaves of plantlets were produced in ventilated vessels which facilitate ex vitro acclimation of plantlets. The plant grown in high oxygen availability was very healthy; respiration and photosynthesis rate were very high with direct effect on plant growth. Similar report by many researchers revealed that rising O_2 supply is apparently balanced by increasing O_2 consumption, that is, mitochondrial respiration (Rolletschek et al., 2005a). In the absence of oxygen, the mitochondrial ATP supply will be inhibited because oxygen is the terminal electron acceptor in the respiratory chain. Hence, it is not surprising that the imposition of hypoxia leads to a rapid decrease in both the availability of ATP and biosynthetic fluxes Geigenberger (2003); Greenway and Gibbs (2003); Rolletscheket al. (2003), it was also reported bymany authors (Chang et al., 2000; Klok et al., 2002; Liu et al., 2005) that oxygen affects gene

Picture 2. Effect of aerophilic and micro-aerophilic conditions on growth.

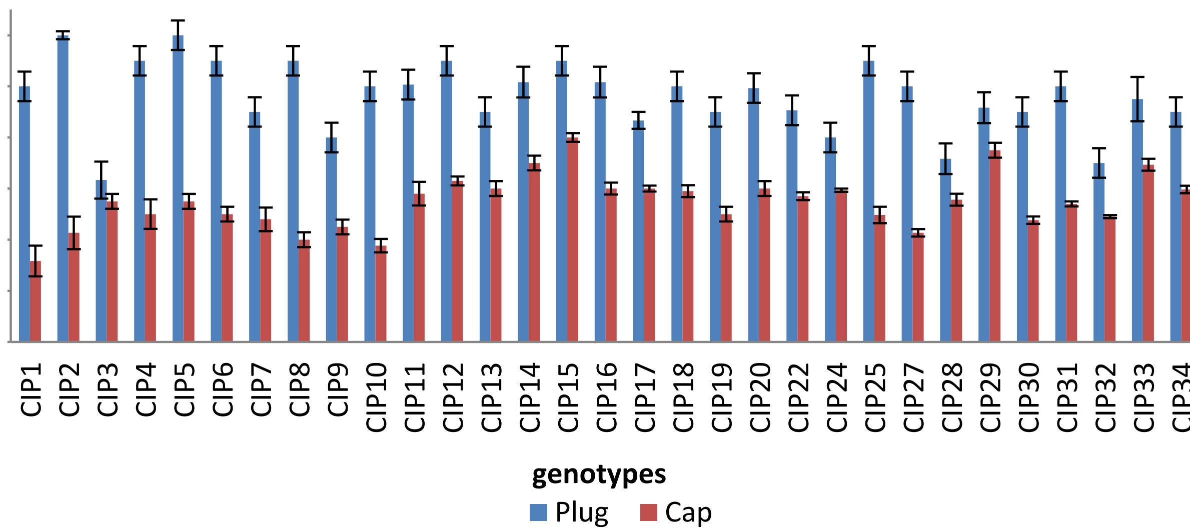

Figure 3. Effect of aerophilic and micro-aerophilic conditions on plant height of 34-CIP potato genotypes.

expression more generally and thus affect the plant growth. It was observed in the present study that plugcovered plant produced large number of root than the plug grown plants which can directly affect the plant growth. Similar observation was obtained by many other researchers. Cherif et al. (1997), Bhattarai et al. (2006), and Acuña et al. (2008) reported that aeration is one of the important factors that influence root and plant growth. Plant cells require oxygen for division and function. If rooting medium has oxygen deficiency, plants will be severely injured or dead in limited time.

Respiration requires oxygen to produce energy for shoot and root growth and also helps in ion absorption. Metabolic processes like cell division, water movement into roots and mineral uptake can be prohibited by root oxygen scarcity creating changes in root system morphology; also, roots will die after disturbance of absorbing water and ions resulting from lack of satisfactory oxygen reported by Morard and Silvestre (1996) and Caron and Nkongolo (2004). Mobiniet al.(2009) also reported that increasing the level of aeration led to remarkable increase in growing period and delay in physiologic maturity of plant but induced tuber initiation. Ritter et al. (2001) and Factor et al. (2007) reported thatsufficient amount of O_2 concentration was needed to increase tuber yield, dry matter and most of the growth parameters

such as green leaf area index (LAI), grain harvest index (HI) and significantly ratio of root and shoot.

Conclusion

The study thus revealed that temperature of 25°C is the most favorable for multiplication of potato germplasm because at this temperature the growth rate is very fast, irrespective of the variety and time interval but for conservation of in vitro germplasm, 10°C was found optimal. Establishing the proper conditions for slow growth in potato will aid in preserving their germplasm for the purpose of later reintroduction and sustainable use. In micro-areophillic condition, the growth rate was very slow as compared to areophillic which shows its suitability for the conservation of plantlets.

Conflict of Interests

The authors have not declared any conflict of interest.

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