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EFFECT OF DIFFERENT HORMONAL CONCENTRATIONS ON IN-VITRO REGENERATION OF RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Rice (*Oryza sativa* L.) contains carbohydrates, fiber, and vitamins, is a major cash crop of Pakistan. The present study was aimed to evaluate the effect of different hormonal concentrations on the *in-vitro* growth and multiplication of rice seeds. Rice seeds were collected from the local market and were surface sterilized with different concentrations of chlorox i. e. (30, 50 and 50%) and germination percentage was calculated. The maximum (70%) germination was recorded for seeds treated with 50% chlorox. Whereas minimum germination (37.5%) was recorded for seeds treated with 70% chlorox. The seeds were cultured on MS medium supplemented with varying concentrations of Indole acetic acid (IAA) (0.1, 0.2, 0.3, 0.75 and 2mg/l) and Kinetin (KIN) at (0.5, 1, 1.5 mg/l). Maximum stem length (30.5 cm), root number (20), root length (6.6 cm), leaf number (70) and leaf length (7 cm) was recorded for MS medium supplemented with higher concentration of IAA (2mg/l) and KIN (1.5 mg/l). Minimum stem length (6.5 cm), root length (2.5 cm), root number (1) root length (2.5 cm), leaf number (1) and leaf length 1.7cm) was recorded on MS medium supplemented with IAA (0.3 mg/l) and KIN (1.5 mg/l). Present study standardizes efficient regeneration of high yielding and diverse rice cultivars that can be utilized as an efficient tool for genetic transformation in future.

KEY WORDS: In vitro; Hormones; Regeneration; Rice

INTRODUCTION

Rice is important cereal cash crop that is a primary source of carbohydrates and fibers for more than half of the world's population. It is one of most versatile and important cereal crop of Poaceae family. It is cultivated for more than 10,000 years (Sasaki, 2001). In Pakistan it was cultivated on about 3.335 million ha with an average production of 12.63 million tons (FAO, 2020). Rice is the most important staple food in Asia. More than 90% of the world's rice is grown and consumed in Asia, where 60% of the world's population lives. Rice accounts for between 35-60% of the caloric intake of three billion Asians (Guyer *et al.*, 1998; Pravin *et al.*, 2011). Over 150 million hectares of rice are planted annually, covering about 10% of the world's arable land. With the world population estimated to increase from 6.2 billion in the year 2000 to about 8.2 billion in the year 2030, the global rice demand will rise to about 765 million tons, or 533 million tons of milled rice (FAO, 2020). For almost three decades since the Green Revolution, the rice yield growth rate was approximately 2.5% per year. During the 1990s, however, this has

decreased to only 1.1% (Mukherjee *et al.,* 2015; Riveros and Figures, 2000). Asia is the largest producer of rice, with Bangladesh, China, Philippines, Thailand and Vietnams as the leading rice producing nations. In Pakistan rice covered an area of 2.52 million ha, with the production of 5.16 million tons in 1999-2000. It also play important role in our national economy. During 1999-2000 about 1.92 million tons of rice was exported and earn about 465.8 million US$ (Ramesh *et al.,* 2009).

Tissue culture is an experimental approach for basic and applied research. It is an essential component of breeding, biotechnology and genetic improvement of plant. Every plant is considered to be totipotent. The potentiality to readily generate plants from cultured tissue has been considered as a powerful tool for crop improvement and simplest form of genetic engineering (Larkin and Scowcroft, 1981; Sah *et al.,* 2014). Apart from their use as a tool of research, plant tissue culture techniques have in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. Small pieces of tissue (explants) can be used to produce hundreds and thousands of plants in a continuous process (Tariq *et al.,* 2008).

Plant tissue culture is now a well-established technology which has made significant contributions to the propagation and improvement of agricultural crops in general. Understanding of the biological processes that permit the manipulation of in vitro morphogenesis and investigations on various physiological, biochemical and molecular aspects of plant hormones will greatly advance our knowledge and provide information that will help address the issues of in vitro recalcitrance or in vitro plant growth and development (Verma *et al.,* 2011).

A single explant can be multiplied into several thousand plants in relatively short time period and space under controlled conditions, irrespective of the season and weather on a year round basis. Endangered, threatened and rare species have successfully been grown and conserved by micropropogation because of high coefficient of multiplication and small demands on number of initial plants and space. Rice is susceptible to a range of diseases and pests, which annually destroy about 55% of rice crops. The most common diseases are caused by the fungi sheath blight and rice blast, and the stalk borer is a common insect pest. Rice is composed of essential food components, therefore more than two billions people in the globe depend on rice for more than half of the proteins and calories they consume (Khan *et al*., 2000; Upadhyaya *et al.,* 2015). Due to its increasing importance in nutrition and economy, it is now felt that new varieties of rice, having good agronomic characters, should be evolved. Crop improvement through tissue culture techniques is easier and more often in use as compared to conventional plant breeding (Yamada, 1986). Keeping in view the current scenario the study was designed to evaluate the effect of different hormonal concentrations on *in vitro* regeneration of Rice.

MATERIAL AND METHODS

Planting material and sterilization: Rice seeds were collected from local market of Muzaffarabad. Healthy seeds were selected on the basis of their appearance (Figure 1). Standard temperature and photoperiod conditions were provided in the laboratory for the experiment i.e. 25±2 ^{0}C temperature along with 16-18 hours of photoperiod. Sterilization of chambers was done using UV for 15-20 minutes followed by culturing explants sterilization using surface sterilization. After culturing explants were then transferred to growth chamber for germination.

Figure 1. Rice seeds (*Oryza sativa* L.) collected for study.

MS media preparation and sterilization of seeds: MS media was prepared by adding measured concentration of IAA, kinetin stock solutions and 15g sucrose followed by maintaining pH at 5.7- 5.8. Then 3g agar was added to solidify the solution and filled the measuring cylinder up to 500ml distilled water to make half litre media. Then media was boiled and poured into the test tubes followed by sterilizing in the autoclave. Sterilization of glassware and surgical instruments was done using UV light for 15-20 min inside the laminar flow chamber followed by surface sterilization of explants using 30, 50 and 70% sodium hypochlorite (chlorox) and then washing with distilled water thrice.

Culturing of explant for regeneration: Explants were cultured in an antiseptic conditions using laminar flow. For organogenesis seeds were used as explants after washing the seeds were cultured on MS mediumcontaining five (5) different concentrations of hormones as;

IAA= 2.0 mg/, Kinetin= 1.5 /l
IAA= 0.75 mg/l, Kinetin= 0.5 mg/l
IAA= 0.1 mg/l, Kinetin= 0.5 mg/l
IAA= 0.2 mg/l, Kinetin= 1.0 mg/l
IAA= 0.3 mg/l, Kinetin= 1.5 mg/l

All the concentrations were replicated thrice for each medium and were allowed to grow in the growth room at 25±2 ^{o}C with 16-18 hours of photoperiod.

Data analysis: Growth data was collected on daily basis for different parameters viz. number of healthy and contaminated explants, mean, standard deviation and percentages of germination were recorded. While in the drought experiment plant height, number of leaves, leaf length, number of roots and root length was recorded.

RESULTS

Effect of different chlorox concentrations on germination percentage and hormonal concentrations on *in vitro* regeneration of rice (*Oryza sativa* L.) were analyzed. It was observed that after surface sterilization, 24 seeds were inoculated on MS medium and the germination rate was 58.3%. From the total 24 seeds, 10 were contaminated while 14 were germinated (Table 1). It was observed that the higher germination frequency (75%) was observed in using 50% chlorox. Whereas the lowest germination percentage (37.5%) was observed using 70% chlorox and was proved that rice seeds were best germinated using 50% chlorox for sterlization. These results indicated that with the increasing chlorox concentration decreased the viability of explants resulting low germination percentage

(37.5 %).

Effect of different amounts growth regulators of auxin (IAA) and cytokinin (KIN) on regeneration of rice was also investigated. For plant regeneration, different concentration combinations of KIN and IAA were used and found that the media 1 containing IAA 2.0 mg/l, KIN 1.5 mg/l and media 2 containing IAA 0.75 mg/l, IAA 0.5 mg/l showed highest regeneration percentage while media 3containing IAA 0.1 mg/l and KIN 0.5 mg/l and media 4 having IAA 0.2 mg/l, KIN 1.0 mg/l showed low generation percentage comparatively. Whereas media 5 containing IAA 0.3 mg/l, KIN 1.5 mg/l was found the least regeneration percentage (Figure 2).

Table 1. Germination percentage of rice (*Oryza sativa* L.).

Sterilization %	Total seeds	Seedling	Contaminated	Germination %
Chlorox 30 %	8	5	3	62.5 %
Chlorox 50 %	8	6	2	75 %
Chlorox 70 %	8	3	5	37.5 %

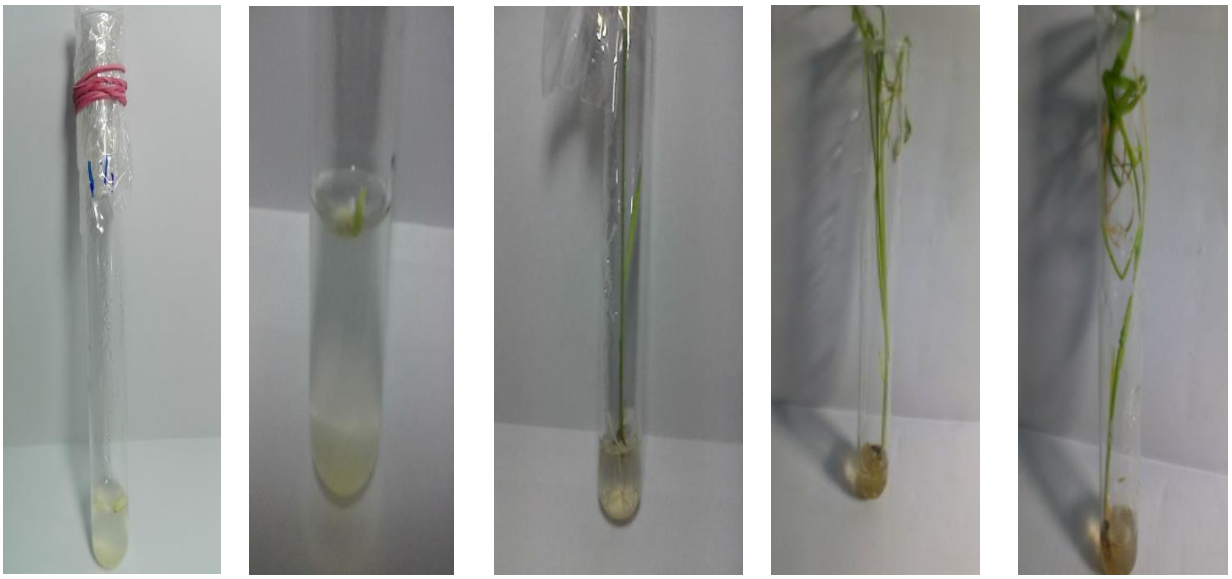

Figure 2. Different stages of regeneration of Rice (*Oryza sativa* L.)

Effect on growth parameters

Stem length (cm): Stem length was recorded when all the explants attained their maximum height and it was evaluated that maximum stem length (30.5 cm) was on media supplemented with IAA 2.0 mg/l, KIN 1.5 mg/l. whereas minimum length (6.5 cm) was on media supplemented with IAA 0.3 mg/l, KIN 1.5 mg/l (Figure 3).

Roots number: Similarly the maximum number of roots (20) were observed in media supplemented with IAA 2.0 mg/l, KIN 1.5 mg/l while minimum number of root (1) was observed in media supplemented with IAA 0.3 mg/l, KIN 1.5 mg/l (Figure 4).

Root length (cm): The medium containing IAA 2.0 mg/l, KIN 1.5 mg/l showed maximum root length that was 7cm while minimum root length was observed in medium containing IAA 0.3 mg/l, KIN 1.5 mg/l that was 2cm (Figure 5).

Number of leaves: Maximum number of leaves (7) was observed on medium comprising of IAA 2.0 mg/l, KIN 1.5 mg/l while minimum number of leaves (1) was observed on medium comprising of IAA 0.3 mg/l, KIN 1.5 mg/l (Figure 6).

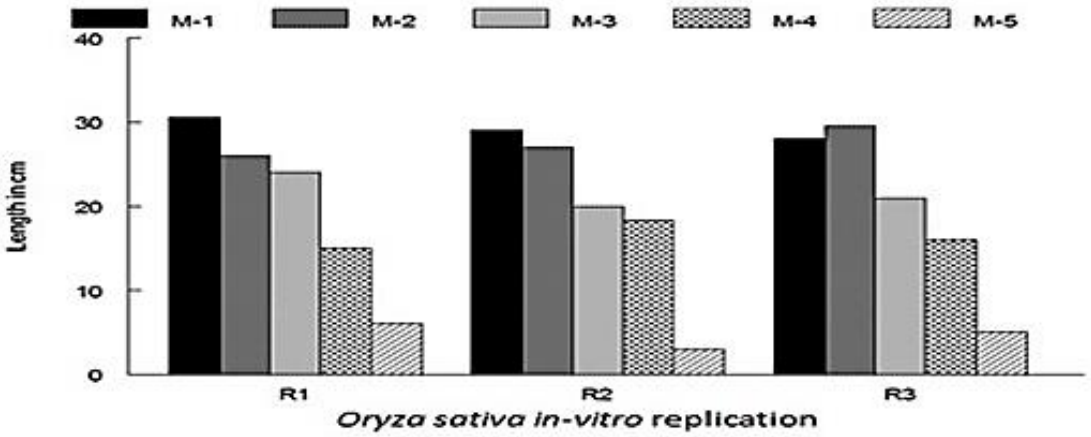

Figure 3. Comparison of different medium concentration on stem length (cm) for regeneration of rice.

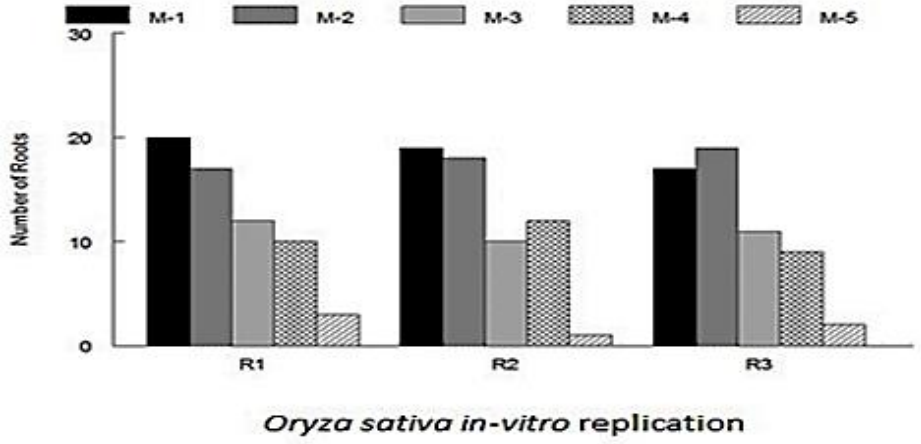

Figure 4. Comparison of different medium concentration on number of roots for regeneration of rice.

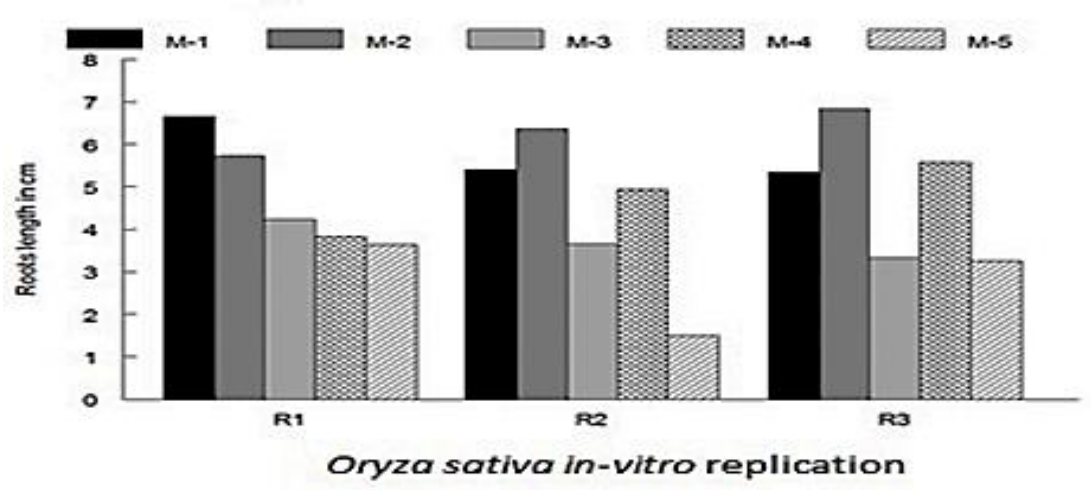

Figure 5. Comparison of different medium concentration on root length (cm) for regeneration of rice.

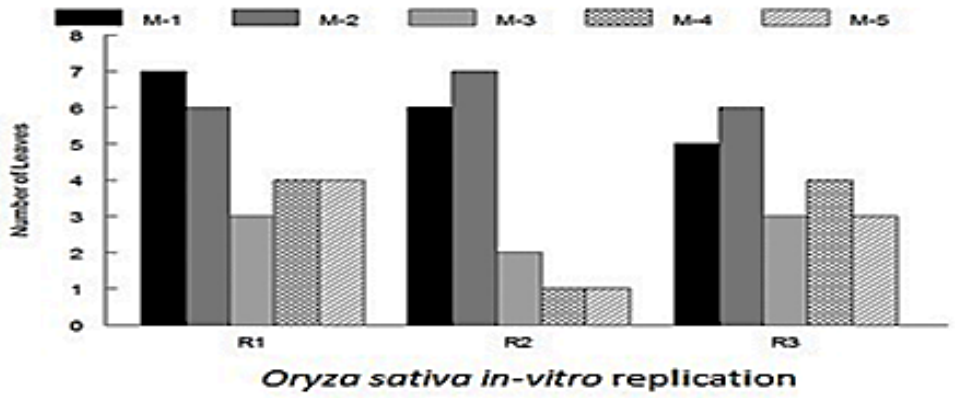

Figure 6. Comparison of different medium concentration on number of leaves for regeneration of rice.

Leaves length (cm): Similarly the medium containing IAA 2.0 mg/l, KIN 1.5 mg/l showed maximum leaf length (7.16 cm) while minimum leaf length 1.7cm was observed using medium containing IAA 0.3 mg/l, KIN 1.5 mg/l. These results indicated that higher concentration of auxin (IAA) and cytokinin (KIN) promotes regeneration frequency in rice (Figure 7).

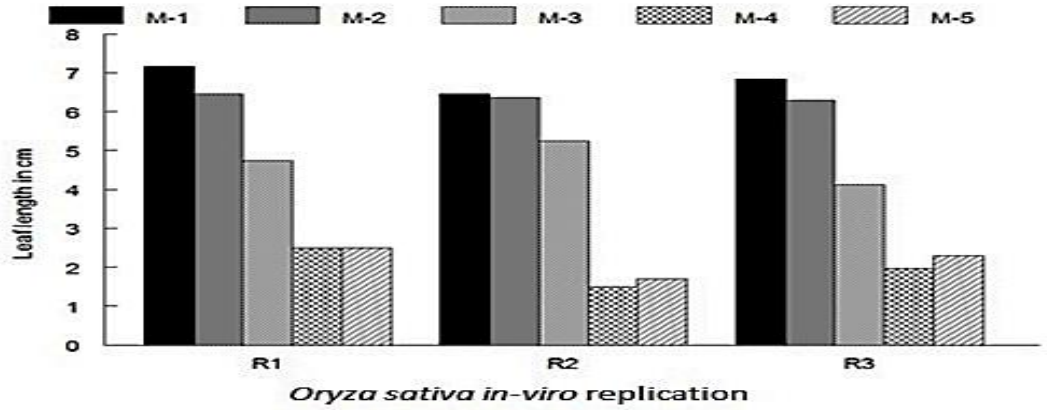

Figure 7. Evaluation of different medium concentration on leaves length for regeneration of rice.

DISCUSSION

In the present *in vitro* study, significant response of different concentrations of hormones were observed germination percentage and different growth parameters and was observed that the 50% concentration of clorox solution was showed maximum germination percentage. Based on results obtained, rice shows higher regeneration frequency on media supplemented with higher concentration of auxin and cytokinin. It was demonstrated that auxin to cytokinin ratio is vital to *in vitro* response of tissue culture. Results indicate that higher level of IAA and KIN promotes rooting and shooting in rice seeds. The maximum stem length (30.5 cm) was observed on media supplemented with IAA 2.0 mg/l and KIN 1.5 mg/l. These results indicated that higher concentration of auxin (IAA) and cytokinin (KIN) promotes regeneration frequency in rice. These results are consistent with the work of Pandey *et al*. (1994) who reported that 2.0 and 3.0 mg L-1 IAA and kinetin produced the most shoots in rice. Verma *et al.* (2011) also reported that 0.5 and 2.0 mg L–1 IAA and kinetin in regeneration medium were beneficial for green plantlet differentiation of rice (*Oryza*

sativa L.). Hormonal regulation depends not only on hormone concentration in plant tissues, but also on tissue susceptibility to growth regulators, which changes depending on stage of plant, organ or tissue development. Thus, the responses evoked by hormones are rarely proportional to their pool (Alam *et al.*, 2012; Ghobeishavi *et al.*, 2015; Gaspar *et al.*, 2003). Sterilization is the major factor, which affects the tissue culture. Chlorox (Sodium hypo chlorite) was used as a surface sterilization agent, played very important role in the germination of seeds (Mahajan and Sharma, 2015; Anand *et al.*, 2014). Clorox 50% was found more suitable for sterilization without browning and inhibition of germination. These results are nearly similar to (Chowdhury *et al.*, 2012; Gonalz et al., 2000; Lee *et al*., 2002) who reported that sterilization of seeds of rice with 45% (v/v) Chlorox was effective.

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