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INVITRO-PLANT REGENERATION FROM MATURED SEED EXPLANT OF LOCAL AND IMPROVED RICE (ORYZA SATIVA L.) VARIETIES

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ABSTRACT

Plant tissue cultures of crops are carried out to produce more crops within a short period of time and as well to overcome the demands of food by the fast growing population. Numerous concerns have been centered towards indica rice, for a reason the study aimed at assessing the growth and if possible yield performance of some local rice varieties using invitro plant regeneration method. The study was conducted to observe the regeneration potential and also to establish a suitable in vitro plantlet regeneration protocol from mature seed explant of four rice varieties viz local rice Variety (Maizabuwa and Mai kwalli) and improved rice variety (Nerica and Faro 44). Maizabuwa was the only variety that successfully regenerated by proliferating shoot and root from the rice seed explant while Faro 44, had root development in the MS media under in-vitro condition. After transplanting, only Maizabuwa successfully germinated from the soil and plantlet growth was observed. No variations was observed in growth responses (plant height (cm), number of tillers, number of leaves and leaf area (cm2)) between in-vitro and soil regenerated (potted in soil) Maizabuwa and Faro 44 rice varieties, whereas variation in growth responses measured between the four varieties of rice directly potted in soil media was observed. Soil potted plants showed some significant differences in growth where Maizabuwa and Nerica varieties indicated to contribute to such variation. The study indicate that seeds of Maizabuwa local rice variety can be suggested to farmers if interested in large scale farming as the variety has shown observable tolerance to in-vitro regeneration and as well potted soil media conditions.

Keywords: Plantlet Regeneration; MS media; Explant; Rice varieties

INTRODUCTION

Rice (Oryza sativa L.), been the most important consumed cereal belonging to the Poaceae family has served as meal for more than 50 % of the growing World population. However, rice production has slowed down and it is estimated that rice production has to be increased 50 % by the year 2025.

In recent years, new rice varieties have been developed by applying conventional breeding to cope with climate change (Mottaleb et al., 2016) but the success rate is not high. Therefore, conventional breeding methods need to be assisted by recent achievements in biotechnology to meet the increasing demand for rice production. Plant tissue culture techniques are prerequisite for successful applications of plant biotechnology and being applied for varietal development of cereal crops including rice in various countries (Dorosieve, 1996; Islam et al., 2014). Among these techniques, the cultures of anther, leaf, root and dehusked seeds are important in rice tissue culture to exploit somaclonal variations, select invitro and produce new lines from genetic transformation. Many protocols have been developed for the invitro regeneration of rice from different explants,such as immature seeds (Hiei and Komari, 2008; Islam et al., 2014), mature seeds (Sah and Kaur, 2013;

Islam et al., 2014; Upadhyaya et al., 2015), leaf (Karthikeyan et al., 2011), shoot apex (Deyetal., 2012), and root (Mandal et al., 2003). Calli induced from subcutellar tissue of mature seeds are the excellent source of cells for in vitro regeneration and for the production of transgenic rice (Wan et al., 2011). Although there have been some successful reports in plant regeneration from indica rice tissue culture, the protocol is not applicable for all rice cultivars.

Plant regeneration is the major outcome of plant tissue culture where somatic embryogenesis and organogenesis are frequently experimented for the regeneration of plants. Organogenesis means formation of organs from the cultured explants. The shoot bud or monopolar structures are formed by manipulating the ratio of cytokinin to auxin in the cultures. In somatic embryogenesis, the totipotent cells may undergo embryogenic pathway to form somatic embryos, which are grown to regenerate whole plants. It was first established in carrots (Daucus carota), where bipolar embryos developed from single cells. The somatic embryogenesis is influenced by herbal extracts, phytohormones, and the physiological state of calli. Plant regeneration-refers to the physiological renewal, repair or replacement of tissue plant.

There is inadequate supply of oxygen to the embryogenic cells that will enhance plant regeneration. Dehydration and death of shoot of plant due to inappropriate removal of calli from the medium of extraction. Various scientific groups working on indica rice species have tried to overcome recalcitrance problem by transforming the mature embryo derived calli (Hiei et al., 1994), immature embryo (Aldemita & Hodges, 1996) and scutellum derived calli of mature seeds (Rashid et al., 1996). Numerous concerns have been centered towards indica rice, for a reason the study aimed at assessing the growth and if possible yield performance of some local rice varieties using invitro plant regeneration method.

The findings of this study will be very useful for producing high frequency callus induction that is the prime step for crop improvement or rapid propagation through bio technological approaches which provide simple in vitro protocol for generating high frequency callus formation and its subsequent regeneration potentiality. Even though these varieties are said to be high yielding their plants are however susceptible to flooding, drought, and low resistance to diseases and pests.

Growth of living plant tissues in a suitable culture medium (in vitro) is known as plant tissue culture. Culture medium is a nutrient medium which contains all essential micro and macro nutrients, carbohydrates, vitamins and hormones. The pH of Culture medium should be 5.5. However, the culture medium differs from species to species. Thus a suitable medium has to be developed to meet the requirement of a plant species. Plant tissue culture includes cell culture, protoplast culture, organ culture, meristem culture etc. The organ culture includes any plant organ which has separate identity such as anther culture, ovule culture, embryo culture and bud culture. The plant part which is used for regeneration is called explant. It may be a cell, a protoplast, a tissue, or an organ. A mass of unorganized regenerated cells in culture medium is called callus (pleural Calli) and suspension of free cells of callus in a liquid medium is known as suspension culture. The regeneration capacity or ability of a plant cell to develop into a whole plant is known as totipotency, which reveals that each cell is capable of giving rise to a complete plant. The cell and tissue cultures lead to regeneration of complete plant. In some species like carrot, and sandalwood

somatic embryos are developed, but in several crops like wheat, rice, barley and tobacco development of both root and shoot takes place from the Calli.

One of the important uses of tissue culture is to utilize mass propagation invitro for conservation of endangered species, as well as species of economic and medicinal value. In view of rapid denudation of forests and other human practices in relation to industry, agriculture and excessive land use, several valuable species are on the verge of extinction. The propagation in vitro through organogenesis or embryogenesis which utilizes only small amount of tissue, has become a powerful tool for increase of individuals. The seeds of endangered as well as other economic valuable plants can also be maintained for a long period without the loss of viability through preservation in ultra-cool temperature, otherwise termed as cryopreservation.

MATERIALS AND METHODS

Study Area

The experiment was carried out in microbiology laboratory and transplanted to the screen house of Biological Sciences Department Garden situated behind science complex, Gombe State University. The sample site is located at a total size of 270 square meters or 2.7 hectares. The garden lies between latitude 10°E 18' and longitude 11° 10' 36.43"E and has an altitude/ elevation of 438-478 m above sea level (figure 1).and mean daily maximum and minimum temperature of 32 0C “89 0F” and 22 0C “71 0F” respectively.

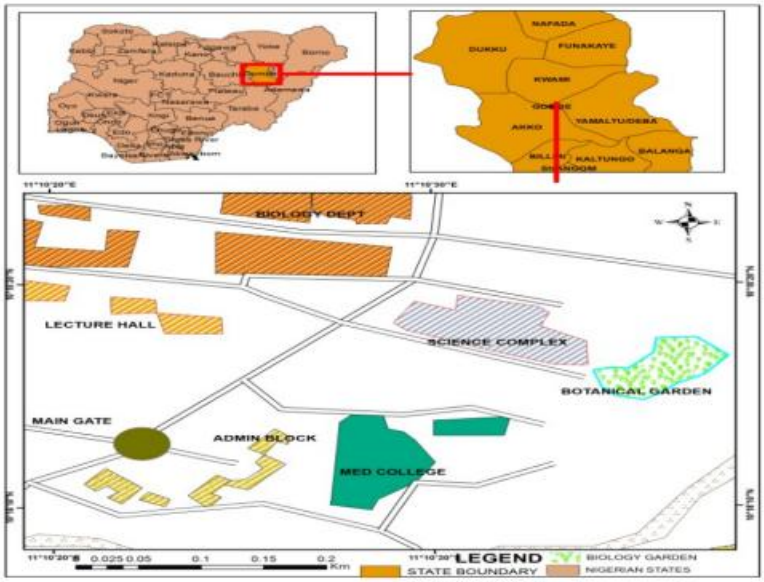

Figure 1: Map of Gombe State University showing Biological Garden. Source: GPS Lab. Geography department Gombe State University

Source of Plant Material

The local plant material was collected from School of Agriculture, Tumu, Akko Local Government in Gombe State latitude 10º 16' 15’N to 10º 17' 7’N and longitude 11º 16' 44’’E to 11º 18' 28’’E. while the improved rice was collected from Department of Applied Ecology ATBU, Bauchi, Bauchi state, latitude 10º 18'50.9724”N and longitude 9º

50'46.6152" E. These was brought to the laboratory unit of department of Biological Sciences, Gombe state university.

The experiment utilized 4 rice varieties of 2 improved rice varieties (Nerica and Faro 44) and 2 local varieties (Maizabuwa and Mai kwalli).

Murashige-Skoog Media (MS) Preparation

MS media are formulated to induce organogenesis, and regeneration of plants in cultured tissues. Media was prepared to be highly enriched with: Inorganic nutrients, Carbon and energy sources, Organic supplements, Growth regulators, Solidifying agents and pH of medium. All components were prepared according to the requirements for adequate plant growth. The medium has to be modified as per the requirement of a species. The culture media developed by Murashige and Skoog (1962) and Gamberg, et al.(1968) were used with some modification.

Seeds Sterilization and Establishment of Culture

The plants were manually dehusked and sterilized with 70 % ethanol for 60 seconds, 2 % sodium hypochlorite for 2 mins with two drops of tween 80 and were rinsed thoroughly with sterilized distilled water under laminar flow cabinet and aseptically placed in the prepared MS media.

In-vitro Plantlet regeneration

Cultured seeds were incubated in a culture room maintained at 26 0C under 16 h/8 h light/dark cycle. After two weeks plantlet was transferred to pot containing garden soil and maintained till maturity.

Data collection

Data were collected three times weekly, parameters collected were: plant height (cm), number of tillers, number of leaves, and leaf area (cm2).

Data Analysis

The Data collected were subjected to analysis of variance using g SPSS version 16.0.

RESULTS

Figure 2 shows in-vitro regenerated plant growth for the four rice varieties where only Maizabuwa and Faro 44 varieties survived. Variation in shoot and root length (cm) after two weeks regeneration.

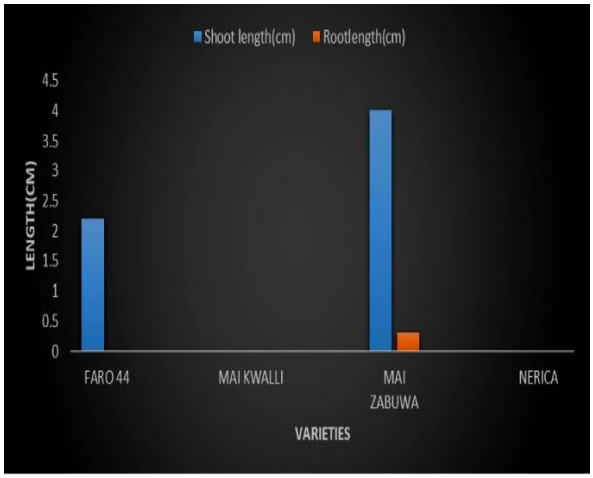

Figure 2: In-vitro regenerated Maizabuwa and Faro 44 rice varieties in the MS media

Growth performance of in vitro and soil planted rice

From the result below, the differences in plant height between in-vitro and soil regenerated rice variety (Maizabuwa) from week one to week three is not significantly different while week four has shown to be significantly different (Table 1).

Number of tillers of in vitro and soil regenerated rice

From the result below, the differences in number of tillers between in-vitro and soil regenerated rice variety (Maizabuwa) in weeks one, two and four were not significantly different while week three is significantly different (Table 2).

Table 1: Plant height (cm) of in vitro and soil regenerated rice

Treatments	Week 1	Week 2	Week 3	Week 4
In-vitro regenerated rice	18.97±3.2	27.33±2.5	31.17±0.76	32.73±0.46
Soil regenerated rice	20.4±4.3	28.83±2.5	32.0±1.0	33.63±0.32
P. values	0.67	0.50	0.32	0.05

Table 2: Number of tillers of in vitro and soil regenerated rice

Treatments	Week 1	Week 2	Week 3	Week 4
In-vitro regenerated rice	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0
Soil regenerated rice	2.0±0.0	1.67±0.58	2.33±0.58	2.0±0.0
P. values	-	0.12	**0.02**	-

Number of leaves of in vitro and soil regenerated rice

The differences in number of leaves between in-vitro and soil regenerated rice variety (Maizabuwa) from week one to week four was observed not to be significantly different (Table 3).

Leaf area (cm^2) of in vitro and soil regenerated rice

The differences in leaf area (cm^2) between in vitro and soil regenerated rice variety (Maizabuwa) from week one to week three is not significantly different while week four is significantly different (Table 4).

Table 3: Number of leaves of in vitro and soil regenerated rice

Treatment	Week 1	Week 2	Week 3	Week 4
In-vitro regenerated rice	3.33±0.58	4.0±0.0	3.0±1.73	5.0±0.0
Soil regenerated rice	3.0±0.0	4.33±0.58	5.0±0.0	5.0±0.0
P. values	0.38	0.38	0.12	-

Table 4: Leaf area (cm^2) of cultured and uncultured rice varieties

Treatment	Week 1	Week 2	Week 3	Week 4
In-vitro regenerated rice	6.69±1.24	13.28±2.16	16.0±1.55	17.97±0.17
Soil regenerated rice	8.68±2.68	14.15±1.87	16.52±1.63	19.09±0.33
P. values	0.31	0.63	0.71	**0.01**

Growth performance in plant height (cm) between the four soil regenerated rice varieties

The variation in plant height (cm) between the four varieties of rice in week one is not significantly different, while from week two to week four is shown to be significantly different (Table 5). Maizabuwa and Nerica were statistically different in plant height (cm) to Faro 44 and Maikwalli in week two. All the varieties were statistically different from each other in plant height (cm) from weeks three to four respectively.

Variation in number of tillers between the four soil regenerated varieties of rice

The variation in number of tillers between the four varieties of rice in week two is significantly different showed mostly to be caused by Maikwalli variety while weeks one, three and four are observed not to be significantly different (Table 6).

Table 5: Variation in plant height (cm) between the four soil regenerated varieties of rice

Varieties	Week 1	Week 2	Week 3	Week 4
Maizabuwa	20.40±4.30^{a}	28.83±2.47^{b}	32.0±1.0^{c}	33.63±0.32^{c}
Faro 44	25.13±4.37^{a}	34.40±1.97^{a}	36.57±0.40^{b}	37.67±0.35^{b}
Mai kwalli	24.60±3.70^{a}	37.33±0.96^{a}	40.57±1.31^{a}	44.70±3.73^{a}
Nerica	17.80±2.62^{a}	27.03±1.50^{b}	28.97±0.67^{d}	29.93±0.40^{d}

Mean with the same alphabet are not significantly different from each other p≤0.05

Table 6: Variation in number of tillers between the four soil regenerated varieties of rice

Varieties	Week 1	Week 2	Week 3	Week 4
Maizabuwa	2.0±0.0	1.67±0.58^{b}	2.33±0.58^{a}	2.0±0.0
Faro 44	2.0±0.0	2.0±0.0^{b}	2.0±0.0^{a}	2.0±0.0
Mai kwalli	2.0±0.0	3.0±0.0^{a}	2.0±0.0^{a}	2.0±0.0
Nerica	2.0±0.0	2.0±0.0^{b}	2.0±0.0^{a}	2.0±0.0

Mean with the same alphabet are not significantly different from each other p≤0.05

Variation in number of leaves between the four soil regenerated varieties of rice

From the result below, variation in number of leaves between the four soil regenerated varieties of rice from week one to week three is not significantly different, while in week four there is no variation (Table 7).

Variation in leaf area (cm^2) between the four soil regenerated varieties of rice

Variation in leaf area (cm^2) (cm^2) between the four soil regenerated varieties of rice in week one is not significantly different, while from week two to week four there is a noticeable significant difference (Table 8).

Table 7: Variation in number of leaves between the four soil regenerated varieties of rice

Varieties	Week 1	Week 2	Week 3	Week 4
Maizabuwa	3.0±0.0^{a}	4.33±0.58^{a}	5.0±0.0^{a}	5.0±0.0
Faro 44	3.33±0.58^{a}	4.67±0.58^{a}	5.67±0,58^{a}	6.0±0.0
Mai kwalli	2.33±0.58^{a}	4.0±0.0^{a}	4.67±0.58^{a}	5.0±0.0
Nerica	2.33±0.58^{a}	4.0±0.0^{a}	4.67±0.58^{a}	5.0±0.0

Mean with the same alphabet are not significantly different from each other p≤0.05

Table 8: Variation in leaf area (cm^2) between the four soil regenerated varieties of rice

Varieties	Week 1	Week 2	Week 3	Week 4
Maizabuwa	8.68 ± 2.68^{a}	14.15 ± 1.87^{b}	16.52 ± 1.63^{bc}	19.09 ± 0.33^{b}
Faro 44	9.47 ± 3.47^{a}	17.85 ± 0.92^{a}	18.60 ± 0.04^{ab}	20.55 ± 1.62^{ab}
Mai kwalli	10.21 ± 1.76^{a}	17.74 ± 2.74^{a}	20.35 ± 1.13^{a}	21.14 ± 0.07^{a}
Nerica	7.28 ± 1.15^{a}	13.04 ± 1.38^{b}	15.57 ± 1.82^{c}	19.01 ± 0.05^{b}

Mean with the same alphabet are not significantly different from each other $p\leq0.05$

DISCUSSION

Different tissue culture strategies are being applied for varietal improvement of cereal yields rice for various nations (Dorosieve, 1996; Islam et al., 2014). Among these strategies, anther culture, protoplast combination, leaf culture, root culture and dehusked seed culture are significant in rice tissue culture to take advantage of soma clonal variety for production of novel rice assortments. In the current review, ideal culture condition is expected for recovery across assortments. Henceforth plant tissue culture framework is crucial for effective yield improvement. The capacity of plant to recover is fundamental for laying out a fruitful plant culture framework. Nonetheless, not all crop species or assortments can recover without any problem. For four assortments of rice, (Mai kwalli and Maizabuwa) and improved (Nerica and Faro 44), just a single assortment prevailed in recovery under in vitro condition utilizing MS media. Development reactions between in-vitro and soil recovered rice assortments in plant tallness, number of tillers, number of leaves and leaf region generally showed no variety, though variety in plant statures, number of tillers, number of leaves and leaf region between the four assortments of rice pruned in soil media was noticed.

Seeds sprouted on MS medium following 4 days and multi week of culture separately. A few quantities of essential turners were framed following fourteen days of culture. Accordingly, it was proposed that MS medium with no chemical enhancement was viewed as awesome as announced in a comparative report by Puhan and Siddiq (2013).

Plant tissue culture strategies are essential for effective utilizations of plant biotechnology and being applied for varietal improvement of grain crops remembering rice for different nations (Dorosieve, 1996; Islam et al., 2014). Among these procedures, the way of life of anther, leaf, root and dehusked seeds are significant in rice tissue culture to take advantage of somaclonal varieties, select invitro and produce new lines from hereditary change. Numerous conventions have been produced for the invitro recovery of rice from various explants, like juvenile seeds (Hiei and Komari, 2008; Islam et al., 2014), mature seeds (Sah and Kaur,2013; Islam et al., 2014; Upadhyaya et al., 2015),leaf (Karthikeyan et al., 2011), shoot pinnacle (Dey et al., 2012), and root (Mandal et al., 2003). Calli initiated from scutellar tissue of mature seeds are the magnificent wellspring of cells for in vitro recovery and for the creation of transgenic rice (Wani et al., 2011). Indica rice assortments are prominently filled in the Mekong Delta district, however they are obstinate to invitro recovery due to unfortunate callus acceptance and recovery productivity. Despite the fact that there have been a few fruitful reports in plant recovery from indica rice tissue culture, the convention isn't relevant for all rice cultivars. These could

likewise be reasons Nerica or maikwalli vaieties couldn't multiply under the MS media culture.The use of amino acid proline in the medium has been reported to have positive effects on regeneration in rice (Ge *et al*., 2006; Shahsavari, 2011). In rice, the rate of shoot regeneration from callus are influenced by the many factors like explants source, genotype, culture conditions and combinations of plant growth regulators, osmotic pressure, and partial desiccation (Venuprasad et al., 2015). In recent years, new rice varieties have been developed by applying conventional breeding to cope with climate change (Mottaleb *et al.,* 2016) but the success rate is not high. Therefore, conventional breeding methods need to be assisted by recent achievements in biotechnology to meet the increasing demand for rice production Shoot proliferation was significant in Faro 44 and Maizabuwa and root was significant only in Maizabuwa, while Mai kwalli and Nerica showed no root or shoot growth.

CONCLUSION

From the experiment in this study, MS media was the only media used, Maizabuwa succeeded in proliferating shoot and root from the rice seed explant while in Faro 44 shoot development was noticeable. Nerica and Maikwalli did not regenerate in the MS media. After transplanting only Maizabuwa successfully germinated from the soil and plantlet growth was observed. Soil potted plants showed some significant differences in growth where Maizabuwa and Nerica varieties indicated to contribute to such variation.

It is recommended that seeds of Maizabuwa local rice variety can be suggested to farmers if interested in large scale farming as the variety has indicated tolerance to cultures and as well potted soil media conditions.

Tissue culture experiment can be done to improve the germination rate of rice seed explant in shorter periods of time.

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