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# In Vitro Callus Induction and Plant Regeneration in Bread Wheat (Triticum aestivum L.) Genotypes from Young leave base

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**Abstract**
Callus initiation and regeneration of plantlets in wheat can be held by different types of explants. Monocots are less responsive to in vitro regeneration, partly due to the fact that in cereals cells and immature or young tissues can respond efficiently to regeneration. Young leave base of three wheat genotypes were cultured on Murashige and Skoog medium supplemented with 2 mg/l of 2, 4-D for callus induction, 1.0 mg/l Kinetin for shoot regeneration and half strength MS media supplemented with 1.0 mg/l indole-3- butyric acid (IBA) for root regeneration. *In vitro* culture response of the genotypes varied from each other and was influenced by the genotypes. Among the genotypes G3 had the highest callusing ability (66.67%) followed by G1 (50.95%). Similarly G3 had the highest shoot and root regeneration frequency of 65.63 % and 86.11 % respectively. Overall, G3 had the highest callus induction, shoot and root regeneration capacity; therefor G3 is a best for transformation.

**Keywords:** *Callus, Bread wheat, In vitro, Regeneration*.

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## Introduction

Bread wheat is one of the most important agricultural commodities grown all over the world. It is an exotic crop cultivated in a wide range of agro-ecology of Ethiopia. Despite the significant dietary and ecological importance, its productivity in Ethiopia is far below its potential which is 2.74 ton $ha^{-1}$ (CSA, 2018). Conventional practices coupled with genetic engineering and plant transformation can lead to a significant increase in wheat production by improving their characteristics and tolerance to various biotic and abiotic stresses.

Callus induction and regeneration of whole plants is required for successful application of tissue culture approaches in crop improvement. Wheat is recalcitrant cereals to in vitro culture (Razzaq *et al*., 2011). Therefore, selection of appropriate genotype for in vitro manipulation is required. Callus induction and regeneration capacity of wheat are influenced by the genotypes, explant source, geographical origin, physiological status of the donor plants, the culture medium and the interactions between them (Ozgen *et al*., 1996). Among these factors, the genotype appears to be important factor influencing the efficiency of culture (Fennel *et al*., 1996; Aydin *et al*., 2011). Explants: Mature and immature embryos (Varshney *et al*., 1999), Coleoptile and Inflorescenses (Benkirane *et al*., 2000), leaves (Zamora and Scott 1983) and anthers (Armstrong *et al*., 1988) were used for callus induction and plant regeneration in wheat. Immature embryo is preferred as explant source for tissue culture and genetic transformation (Gill *et al*., 2014; Yang *et al*., 2015). However, the production of immature embryos throughout the year is a space, time and labour intensive task. Therefore this study is an attempt to test the callusing and regeneration potential of different genotypes from young seedling leave explants.

## Materials and Methods

The experiments were carried out on three bread wheat genotype selected for their high agronomic performance. The experiment was conducted at Tissue Culture laboratory of Mekelle Agricultural Research Center during 2018. A completely randomized design with 40 replications for callus induction, 16 replications for shoot regenerations and 11 replications for root regenerations per genotype was used.

Mature seeds were surface-sterilized for 5 min in 70% ethanol and rinsed with sterile distilled water for five times and kept in 5% sodium hypochlorite supplemented with 1 to 2 drops of tween 20 for 20 minutes, followed by five washes in sterile distilled water. The sterilized seeds were transferred to glass jars of 250 ml capacity

containing 40 ml of solidified basal MS medium (Murashige and Skoog,1962) supplemented with 30 g/L sucrose and 8 g agar. After 5 days, young seedling basal leaves were used for callus induction.

Six explants of basal leaf about 3-4 mm long were aseptically excised and then incubated on MS callus induction medium supplemented with 2 mg/l 2.4-dichlorophenoxyacetic acid (2.4-D), 8 g agar and 30 g sucrose for 5 weeks. For shoot regeneration the callus were transferred into glass jar containing MS basal salt supplemented with 1.0 mg /lKinetin for about 6 weeks. Rooting was initiated on half strength MS media supplemented with 1.0 mg/l indole-3- butyric acid (IBA) for about 5 weeks. The culture media were refreshed every 16 to 21 days. Analysis of variance and least significant-difference tests were done using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS) software 9.0 (SAS Institute Inc., 2002).

## Result and Discussion

The genotypes differed significantly in their ability to callus induction, shoot and root regeneration (Table 1). Indicating the presence of genetic variation among the tested genotypes to callus induction, shoot and root regeneration using young seedling leaves. Similar findings were reported in wheat by different authors (Huimin *et al*., 2012**;** .chen *et al*., 1980; Kopertekh and Stribnaya 2003). Callus induction was observed following five weeks of culture. The amount and type of callus varied with genotypes. Callus induction frequencies varied from 41.88 % to 66.67% (Table 1). Among the genotypes G3 had the highest callusing ability (66.67%) followed by G1 (50.95%). These results confirmed that callus induction was greatly influenced by the genotypes, which is in agreement with reports of callus induction in wheat (Farshadfar *et al*., 2012; Imran and Abdul 2017).

Shoot regeneration frequencies were significantly different among the genotypes varied from 46.87 % to 65.95%. G3 exhibited higher shoot regeneration potential (65.63 %) followed by G1 (58.34%). In contrast, G2 (46.87) possessed lower values. Similar findings were reported that different wheat genotypes had different shoot regeneration frequencies (Ashraf *et al*., 2012). Moreover root regeneration frequency was significantly different among the three studied genotypes. All the genotypes had the height rooting frequencies ranging from 72.22 to 86.11% with mean rooting frequency of 78.7% (Table 1). These genotypic differences may be related to the variations in the endogenous hormone levels between the tested genotypes. The result demonstrates the strong influence of genotype on callus induction and plant regeneration.

In general the genotypes vary in their tissue culture response. All the three genotypes showed callus, shoot and root regeneration, showing that young seedling leave explant is an alternative explant for callus induction, shoot and root regeneration. Overall, among the three genotypes tested G3 had the highest callus induction, shoot and root regeneration capacity.

Table 1. Callus induction and plantlet regeneration

| Genotype | Callus induction frequencies (%) | Shoot regeneration (%) | Rooting (%) |
|---|---|---|---|
| G1(MEX94.27.1.20/3/SOKOLL//ATTILA/3 *BCN/4/ZAFIR-3) | 50.95c | 58.34b | 72.22b |
| G2(SERI.1B//KAUZ/HEVO/3/AMAD/4/ESDA/SHWA//BCN) | 41.88b | 46.87c | 77.78ab |
| G3(SERI.1B//KAUZ/HEVO/3/AMAD/4/ATTILA//PSN/BOW/3/ATTILA | 66.67a | 65.63a | 86.11a |
| Mean | 53.17 | 56.95 | 78.70 |

 

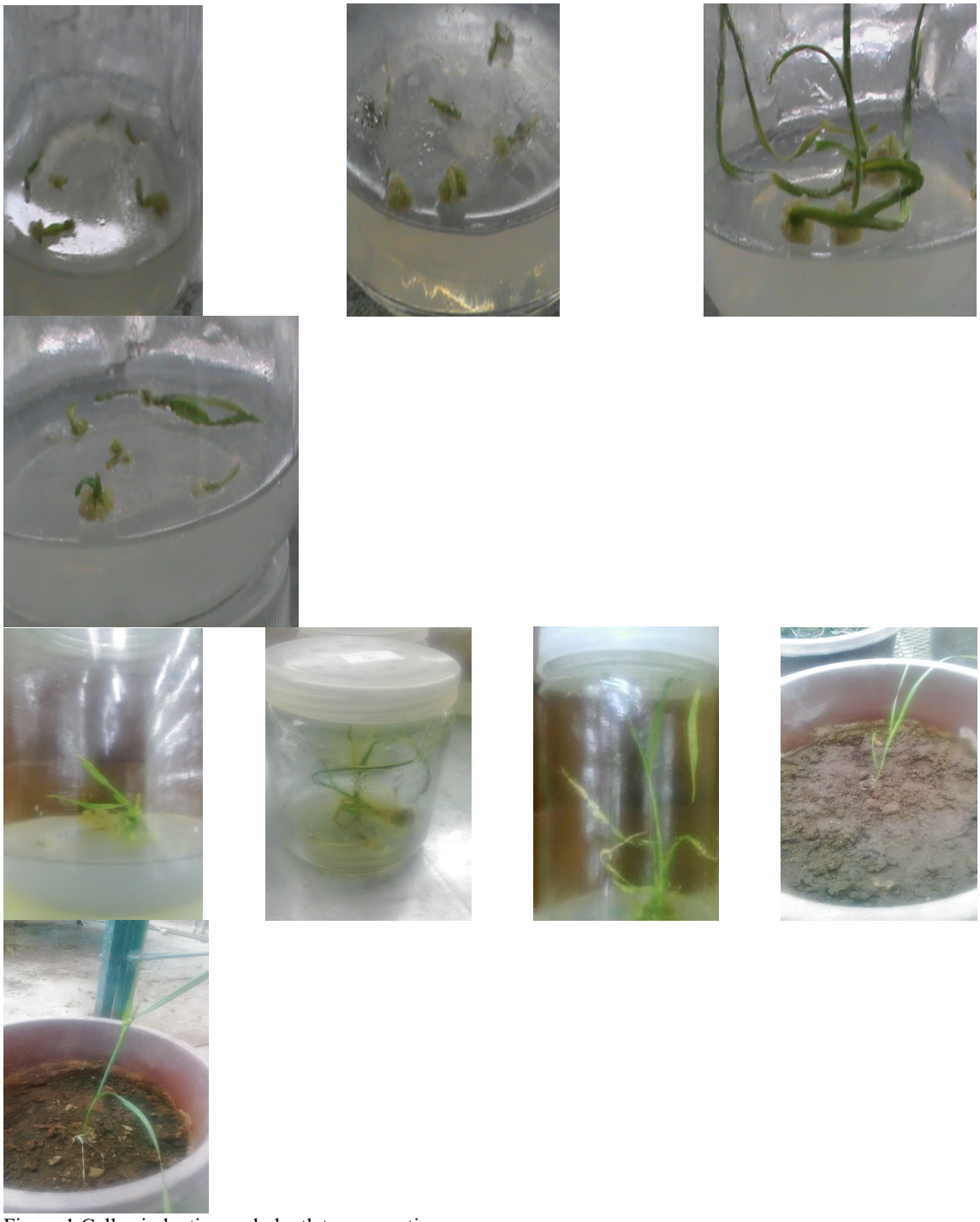

Figure 1 Callus induction and plantlet regeneration

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