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An efficient Regeneration Protocol for *in vitro* Direct Organogenesis in Einkorn *(Triticum monococcum L.)* Wheat

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ABSTRACT
Coleoptile, leaf, and root explants of the einkorn (*Triticum monococcum* ssp. *monococcum*) were cultured *in vitro* to obtain an efficient plant regeneration protocol through direct shoot formation by using different combinations and concentrations of various plant growth regulators. A total of 180 different auxin and cytokinin combinations were tested for regeneration. Shoot formation was not observed with the root and leaf explants. Shoot formation was obtained only from the coleoptile explants, with a mean of 1.20±0.24 shoots/explant and 86.60% of shoot formation frequency and with a 1.20±0.53 shoots/explant and 80.00% shoot formation frequency on medium supplemented with 0.5 mg L^{-1} TDZ and 1 mg L^{-1} TDZ plus 1 mg L^{-1} NAA, respectively. The shoots were subcultured on the MS medium containing the most effective hormonal combination concurrently continued to shoot and root formation for 45 days. It is noteworthy that 3.66±0.66 shoots per explant were induced by MS, which contained 1 mg L^{-1} TDZ plus 1 mg L^{-1} NAA and 2.0 mg L^{-1} KIN plus 0.5 mg L^{-1} NAA for 45 days. Of the different auxin concentrations tested for rooting, 2.0 mg L^{-1} IAA was predominant, with the greatest number of roots (12.33±0.88) produced per regenerated shoot. Finally, these well-developed plantlets were acclimatized with a 100% success rate and were transferred to the ex vitro conditions. A highly efficient regeneration protocol for einkorn wheat was developed using somatic tissue as an explant source for the first time.

Keywords: Coleoptile, Direct regeneration protocol, Tissue culture, Einkorn wheat (*Triticum monococcum* ssp. *monococcum*)

1. Introduction

Micropropagation is used commercially worldwide, but the capacity of plant regeneration and somatic organogenesis varies greatly among species (Bidabadi & Jain 2020). Wheat includes more than 20 cultivated species (Goncharov 2011), however, *in vitro* plant regeneration ability of many wheat species has not been studied until recently. *Triticum monococcum* ssp. *monococcum* (einkorn) wheat, diploid ancestral wheat, intervened in the spread and rise of agriculture for several thousand years until more productive polyploid wheat was replaced with it (Nesbitt & Samuel 1996). But nowadays, the renewed interest in studies related to this cereal is on the rise because of its putative low allergenicity, disease resistance properties, and lower gluten but higher lutein and protein content (Hidalgoa et al. 2006; Özgen et al. 2017). Moreover, it has been recently included in modern wheat breeding programs, as donors of stress resistance genes (Nevo 2011; Login & Reif 2014; Alikina et al. 2016).

Improving crops to create genetic variability and increase the number of desirable germplasms is dependent on the establishment of a highly regenerative tissue culture system for many plant species, particularly cereals. Many factors can affect this system, such as culture medium, growth conditions, genotypes, and explant types. Currently, in tissue culture studies on wheat and other cereal crops, immature-mature embryos and inflorescences have been traditionally used as the most suitable explant source and the regeneration capacity of plants has been reported with varying degrees of success (Benlioğlu & Birsin 2017).

In comparison to other wheat tissues, immature zygotic embryos are the most commonly and efficiently used explants for plant regeneration in hexaploid bread wheat, tetraploid durum wheat, and only a few numbers of studies that have been conducted on diploid wheats (Miroshnichenko et al. 2017). The standard technique entails the cultivation of immature tissues on 2,4-D-containing media in the dark, followed by plant differentiation in the light on media devoid of phytohormones (Fennell et al. 1996; Tama´s et al. 2004; Chauhan et al. 2007; Miroshnichenko et al. 2016). However, this conventional protocol is often ineffective for many wheat genotypes due to the inability to regenerate entire plants on a regular basis. Moreover, the cultivation of donor plants to obtain embryos involves the expenditure of much time and money.

Although using somatic tissues as an explant source makes it possible to obtain a great amount of material regardless of these short-comings, ventures to determine a reliable plant regeneration protocol using somatic cell cultures for diploid wheat species did not produce positive results (Alikina et al. 2016). Similarly, no shoot differentiation was observed in somatic cell cultures of tetraploid and hexaploid wheat genotypes (Lazar et al. 1983; Bi & Wang 2008; Özgen et al. 2017). Therefore, even after many years of research, especially in the genotypes of wheat, screening of germplasms *in vitro* response is very important for biotechnological applications.

The objective of this study was to investigate a highly effective *in vitro* regeneration protocol for the einkorn wheat via adventitious shoot formation from the root, coleoptile, and leaf explants cultured on the MS medium containing different combinations and concentrations of plant growth regulators for the first time. Direct shoot formation from somatic tissue is a remarkable feature of this regeneration protocol.

2. Material and Methods

2.1. Plant materials and growth condition

Seeds of the einkorn (*Triticum monococcum* ssp. *monococcum*) wheat were collected from İhsangazi / Kastamonu, Turkey in 2014-2015. Seeds were disinfected with 100 ml distilled water containing 5 drops of Tween20 (Merck, Darmstadt, Germany) for 1 min, then sterilized with 40% commercial bleach (4.6% NaClO; Domestos, Istanbul, Turkey) for 15 min, and finally washed three times with sterile dH_2O (Örgeç et al. 2018). Seeds (20 seeds / 100 mm×15 mm Petri dish) were cultured on the MS (Duchefa-Haarlem, Netherlands) medium (pH: 5.8), (Murashige & Skoog 1962) containing 2.5% (w/v) sucrose (Merck Darmstadt, Germany) and 0.75% (w/v) agar (Duchefa-Haarlem, Netherlands). The seeds were grown for germination under a 16/8 h photoperiod at 24±2 ºC (climate room conditions) for 10 days.

2.2. Shoot regeneration

Five to eight mm segments of the leaf, coleoptile, and root sliced from ten-days old germinated seedlings were cultured on the MS medium containing different concentrations (from 0.5 to 3 mg L^{-1}) of IAA (indole acetic acid) (Duchefa-Haarlem), NAA (α-naphthalene acetic acid) or 2.4-D (2.4-dichloro phenoxy acetic acid) (Sigma-Aldrich, Steinhem, Germany) and combined with TDZ (thidiazuron) (Duchefa-Haarlem), KIN (kinetin) or BAP (6-benzyl amino purine) ranging from 0.5 to 3 mg L^{-1}. The mean number of shoots/explant and the percentage (%) of developing shoots were enrolled 15 days after the culture grown under climate room conditions (16 h light:8 h dark photoperiod at 23±2 °C), respectively. After a period of 15 days, plantlets were transferred to MS which contained the best response hormone combination to obtain more shoots and observed for up to 45 days. The data were recorded continuously.

2.3. *Rooting of the shoots, hardening, and acclimatization*

Forty-five days old shoots were transferred to MS medium supplemented with IAA (ranging from 0.5 to 5 mg L^{-1}) to observe root formation for 30 days. After 30 days, the mean numbers of roots/explant were recorded. Then, well-developed plantlets were transferred to Magenta vessels (77 mm × 77 mm × 97 mm) containing a mixture of vermiculite and soil (1:2) for acclimatization. The plantlets were kept in the climate room for 1 week. After 1 week, the plantlets were transferred to room conditions.

2.4. *Statistical analysis*

The data were statistically analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Differences in means ± SD (standard deviation) were analyzed using Duncan's multiple range test at $P<0.05$.

3. Results and Discussion

This study provided an efficient protocol for the direct formation of shoots from different explants, the first as such report for einkorn wheat. The traditional method for wheat propagation is using an immature and mature embryo as an explant source. Several studies have been conducted to determine the indirect regeneration protocol from immature and mature embryo cultures in the einkorn wheat (Yang et al. 2015; Alikina et al. 2016; Miroshnichenko et al. 2016). However, there are no reports on einkorn cultures in which improvement in direct shoot production using somatic tissues has been achieved. For this reason, two sets of experiments were carried out. In the first set, the regeneration capacity of three different explant sources (leaf, coleoptile, and root) cultured on the MS medium containing various concentrations of BAP, KIN, or TDZ, in combination with NAA, 2.4-D, or IAA ranging from 0.5 to 3 mg L^{-1} were obtained. In the second set, the most effective hormone combinations were selected to increase the number of shoots.

A total of 180 different hormone combinations were tested for regeneration and variable growth rates were recorded. Control treatments without PGRs produced no shoots. In all tested hormone combinations, shoot formation was not observed with leaf

and root explants. It was observed that coleoptile was the best as an explant source referring to both the percentages of explants forming shoots and the mean number of shoots/explant with TDZ (Table 1 and Figure 1). The TDZ/NAA combination was more effective than the TDZ/IAA or TDZ/2.4-D combination for shoot propagation in this study. When explants were cultured on MS containing KIN or BA in combination with NAA or 2.4-D, the frequency of formation and the number of shoots were found at approximately the same levels. Optimum shoot formation was detected from coleoptile explants, with a mean of 1.20±0.24 shoots/explant and 86.60% shoot formation percentage and with a 1.20±0.53 shoots/explant and 80% shoot formation percentage on medium supplemented with 0.5 mg L^{-1} TDZ and 1 mg L^{-1} TDZ+1 mg L^{-1} NAA, respectively (Table 1).

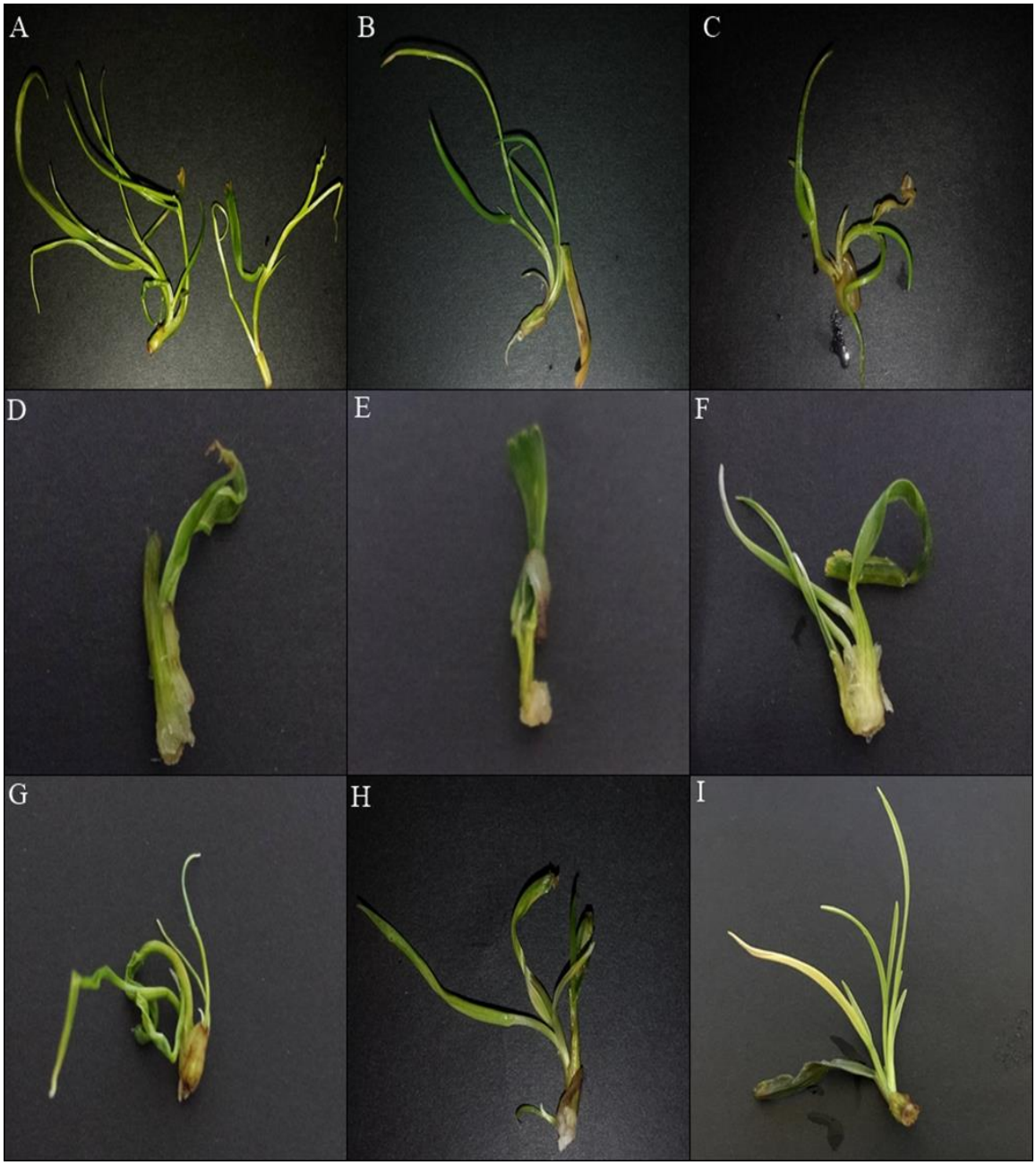

Figure 1- The effects of various concentrations of BA, KIN or TDZ, in combination with NAA, 2,4-D or IAA ranging from 0.5 to 3 mg L^{-1} on shoot formation (A) 0.5 mg L^{-1} TDZ. (B) 1 mg L^{-1} KIN. (C) 2 mg L^{-1} BA+0.5 mg L^{-1} IAA. (D) 2 mg L^{-1} TDZ+1 mg L^{-1} 2.4-D. (E) 0.5 mg L^{-1} KIN+1 mg L^{-1} 2.4-D (F) 3 mg L^{-1} BA+2 mg L^{-1} 2.4-D. (G) 1 mg/l TDZ+1 mg/l NAA (H) 2.0 mg/l KIN+0.5 mg/l NAA. (I) 0.5 mg/l BA+2.0 mg NAA

Table 1- Shoot regeneration from coleoptile explants cultured on MS medium containing different combinations of KIN, TDZ or BA with NAA, 2.4 D or IAA. Mean-values with the same letters within vertical columns are not significantly different (P>0.05). Control means are with no PGR treatment

PGRs (mg L^{-1})	*Mean number of shoots/explant*	*Shoot formation percentage (%)*	*PGRs (mg L^{-1})*	*Mean number of shoots/explant*	*Shoot formation f percentage (%)*	*PGRs (mg L^{-1})*	*Mean number of shoots/explant*	*Shoot formation percentage (%)*
Control	-	-		-	-		-	-
TDZ – IAA			***KIN – IAA***			***BA – IAA***		
0.5 – 0	**1.20±0.24^{a}**	**86.60**	0.5 – 0	0.89±0.38^{abcde}	86.60	0.5 – 0	0.64±0.24^{bcdefghı}	46.60
1.0 – 0	0.78±0.23^{abcdefg}	60.00	1.0 – 0	0.93±0.34^{abcd}	73.30	1.0 – 0	0.64±0.29^{bcdefghı}	40.00
2.0 – 0	0.93±0.38^{abcd}	66.60	2.0 – 0	0.87±0.30^{abcde}	73.30	2.0 – 0	0.78±0.21^{abcdefg}	53.30
3.0 – 0	0.82±0.27^{abcdef}	73.30	3.0 – 0	0.71±0.22^{bcdefgh}	66.60	3.0 – 0	0.62±0.25^{bcdefghı}	46.60
0.5 – 0.5	0.73±0.23^{abcdefg}	60.00	0.5 – 0.5	0.40±0.22^{efghı}	40.00	0.5 – 0.5	0.73±0.11^{abcdefg}	66.60
1.0 – 0.5	0.80±0.20^{abcdef}	66.60	1.0 – 0.5	0.87±0.30^{abcde}	60.00	1.0 – 0.5	0.67±0.50^{bcdefgh}	53.30
2.0 – 0.5	0.73±0.61^{abcdefg}	53.30	2.0 – 0.5	0.33±0.11^{fghı}	33.30	2.0 – 0.5	0.87±0.30^{abcde}	60.00
3.0 – 0.5	0.73±0.12^{bcdefg}	60.00	3.0 – 0.5	0.40±0.22^{efghı}	33.30	3.0 – 0.5	1.00±0.40^{abc}	60.00
0.5 – 1.0	0.60±0.20^{bcdefghı}	60.00	0.5 – 1.0	0.53±0.11^{cdefghı}	46.60	0.5 – 1.0	0.53±0.11^{cdefghı}	53.30
1.0 – 1.0	0.73±0.12^{abcdefg}	60.00	1.0 – 1.0	0.47±0.46^{defghı}	20.00	1.0 – 1.0	0.47±0.11^{defghı}	46.60
2.0 – 1.0	0.60±0.20^{bcdefghı}	60.00	2.0 – 1.0	0.73±0.11^{abcdefg}	33.30	2.0 – 1.0	0.33±0.11^{fghı}	33.30
3.0 – 1.0	0.60±0.20^{bcdefghı}	60.00	3.0 – 1.0	0.20±0.11^{hı}	20.00	3.0 – 1.0	0.47±0.30^{defghı}	40.00
0.5 – 2.0	0.73±0.12^{abcdefg}	60.00	0.5 – 2.0	0.33±0.23^{fghı}	26.60	0.5 – 2.0	0.67±0.23^{bcdefgh}	60.00
1.0 – 2.0	0.87±0.50^{abcde}	53.30	1.0 – 2.0	0.33±0.30^{fghı}	33.30	1.0 – 2.0	0.20±0.11^{hı}	20.00
2.0 – 2.0	0.73±0.23^{abcdefg}	73.30	2.0 – 2.0	0.40±0.20^{efghı}	26.60	2.0 – 2.0	0.53±0.11^{cdefghı}	46.60
3.0 – 2.0	0.67±0.12^{bcdefgh}	73.30	3.0 – 2.0	0.27±0.23^{ghı}	26.60	3.0 – 2.0	0.73±0.22^{abcdefg}	53.30
0.5 – 3.0	0.67±0.46^{bcdefgh}	53.30	0.5 – 3.0	0.40±0.20^{efghı}	40.00	0.5 – 3.0	0.53±0.11^{cdefghı}	53.30
1.0 – 3.0	0.80±0.34^{abcdef}	66.60	1.0 – 3.0	0.47±0.23^{defghı}	40.00	1.0 – 3.0	0.20±0.11^{hı}	20.00
2.0 – 3.0	0.73±0.41^{abcdefg}	53.30	2.0 – 3.0	0.53±0.11^{cdefghı}	40.00	2.0 – 3.0	0.40±0.20^{efghı}	46.60
3.0 – 3.0	0.80±0.20^{abcdef}	53.30	3.0 – 3.0	0.73±0.30^{abcdefg}	46.60	3.0 – 3.0	0.40±0.20^{efghı}	40.00

Table 1 (Continue)- Shoot regeneration from coleoptile explants cultured on MS medium containing different combinations of KIN, TDZ or BA with NAA, 2.4 D or IAA. Mean-values with the same letters within vertical columns are not significantly different (P>0.05). Control means are with no PGR treatment

PGRs (mg L^{-1})	*Mean number of shoots/explant*	*Shoot formation percentage (%)*	*PGRs (mg L^{-1})*	*Mean number of shoots/explant*	*Shoot formation f percentage (%)*	*PGRs (mg L^{-1})*	*Mean number of shoots/explant*	*Shoot formation percentage (%)*
Control	-	-		-	-		-	-
TDZ – 2,4-D			***KIN – 2,4-D***			***BA – 2,4-D***		
0.5 – 0.5	0.80±0.20^{abcdef}	73.30	0.5 – 0.5	0.67±0.31^{bcdefgh}	53.30	0.5 – 0.5	0.33±0.12^{fghı}	33.30
1.0 – 0.5	0.53±0.23^{cdefghı}	46.60	1.0 – 0.5	0.60±0.20^{bcdefghı}	60.00	1.0 – 0.5	0.67±0.61^{bcdefgh}	53.30
2.0 – 0.5	0.67±0.31^{bcdefgh}	60.00	2.0 – 0.5	0.60±0.20^{bcdefghı}	60.00	2.0 – 0.5	0.47±0.42^{defghı}	46.60
3.0 – 0.5	0.60±0.20^{bcdefghı}	60.00	3.0 – 0.5	0.53±0.31^{cdefghı}	53.30	3.0 – 0.5	0.40±0.20^{efghı}	40.00
0.5 – 1.0	0.67±0.42^{bcdefgh}	60.00	0.5 – 1.0	0.73±0.12^{abcdefg}	73.30	0.5 – 1.0	0.40±0.20^{efghı}	40.00
1.0 – 1.0	0.87±0.12^{abcde}	86.60	1.0 – 1.0	0.67±0.12^{bcdefgh}	66.60	1.0 – 1.0	0.67±0.31^{bcdefgh}	60.00
2.0 – 1.0	0.93±0.12^{abcd}	86.60	2.0 – 1.0	0.53±0.12^{cdefghı}	53.30	2.0 – 1.0	0.47±0.12^{defghı}	40.00
3.0 – 1.0	0.73±0.23^{abcdefg}	66.60	3.0 – 1.0	0.60±0.40^{bcdefghı}	53.30	3.0 – 1.0	0.33±0.23^{fghı}	33.30
0.5 – 2.0	0.73±0.12^{abcdefg}	66.60	0.5 – 2.0	0.40±0.40^{efghı}	40.00	0.5 – 2.0	0.20±0.00^{hı}	20.00
1.0 – 2.0	0.67±0.23^{bcdefgh}	66.60	1.0 – 2.0	0.33±0.31^{fghı}	33.30	1.0 – 2.0	0.60±0.20^{bcdefghı}	60.00
2.0 – 2.0	0.67±0.31^{bcdefgh}	66.60	2.0 – 2.0	0.60±0.00^{bcdefghı}	60.00	2.0 – 2.0	0.53±0.12^{cdefghı}	53.30
3.0 – 2.0	0.53±0.12^{cdefghı}	53.30	3.0 – 2.0	0.47±0.31^{defghı}	46.60	3.0 – 2.0	0.93±0.12^{abcd}	80.00
0.5 – 3.0	0.67±0.23^{bcdefgh}	66.60	0.5 – 3.0	0.60±0.20^{bcdefghı}	60.00	0.5 – 3.0	0.60±0.00^{bcdefghı}	60.00
1.0 – 3.0	0.73±0.12^{abcdefg}	73.30	1.0 – 3.0	0.33±0.12^{fghı}	33.30	1.0 – 3.0	0.27±0.31^{ghı}	20.00
2.0 – 3.0	0.47±0.12^{defghı}	46.60	2.0 – 3.0	0.40±0.00^{efghı}	40.00	2.0 – 3.0	0.67±0.12^{bcdefgh}	53.30
3.0 – 3.0	0.13±0.12^{ı}	20.00	3.0 – 3.0	0.47±0.12^{defghı}	46.60	3.0 – 3.0	0.60±0.00^{bcdefghı}	60.00
TDZ – NAA			**KIN – NAA**			**BA - NAA**		
0.5 – 0.5	0.33±0.12^{fghı}	33.30	0.5 – 0.5	0.67±0.12^{bcdefgh}	66.60	0.5 – 0.5	0.67±0.12^{bcdefgh}	66.60
1.0 – 0.5	1.00±0.20^{abc}	66.60	1.0 – 0.5	0.73±0.31^{abcdefg}	73.30	1.0 – 0.5	0.87±0.23^{abcde}	73.30
2.0 – 0.5	0.73±0.12^{abcdefg}	66.60	2.0 – 0.5	0.93±0.12^{abcd}	93.30	2.0 – 0.5	0.67±0.12^{bcdefgh}	66.60
3.0 – 0.5	0.47±0.12^{defghı}	46.60	3.0 – 0.5	0.27±0.46^{ghı}	26.60	3.0 – 0.5	0.80±0.20^{abcdef}	66.60
0.5 – 1.0	1.07±0.12^{ab}	86.60	0.5 – 1.0	0.47±0.12^{defghı}	46.60	0.5 – 1.0	0.67±0.23^{bcdefgh}	66.60
1.0 – 1.0	**1.20±0.53^{a}**	80.00	1.0 – 1.0	0.47±0.12^{defghı}	46.60	1.0 – 1.0	0.60±0.20^{cdefghı}	60.00
2.0 – 1.0	0.67±0.12^{bcdefgh}	66.60	2.0 – 1.0	0.53±0.31^{cdefghı}	60.00	2.0 – 1.0	0.87±0.12^{abcde}	73.30
3.0 – 1.0	0.67±0.12^{bcdefgh}	66.60	3.0 – 1.0	0.80±0.20^{abcdef}	60.00	3.0 – 1.0	0.33±0.31^{fghı}	33.30
0.5 – 2.0	0.67±0.12^{bcdefgh}	80.00	0.5 – 2.0	0.33±0.12^{fghı}	26.60	0.5 – 2.0	1.00±0.53^{abc}	66.60
1.0 – 2.0	0.80±0.40^{abcdef}	60.00	1.0 – 2.0	0.67±0.31^{bcdefgh}	60.00	1.0 – 2.0	0.53±0.12^{cdefghı}	53.30
2.0 – 2.0	0.67±0.12^{bcdefgh}	60.00	2.0 – 2.0	0.53±0.12^{cdefghı}	53.30	2.0 – 2.0	0.60±0.20^{cdefghı}	60.00
3.0 – 2.0	0.87±0.12^{abcde}	80.00	3.0 – 2.0	0.27±0.23^{ghı}	26.60	3.0 – 2.0	0.93±0.12^{abcd}	73.30
0.5 – 3.0	0.73±0.12^{abcdefg}	73.30	0.5 – 3.0	0.60±0.20^{bcdefghı}	53.30	0.5 – 3.0	0.53±0.31^{cdefghı}	53.30
1.0 – 3.0	0.67±0.12^{bcdefgh}	66.60	1.0 – 3.0	0.53±0.12^{cdefghı}	53.30	1.0 – 3.0	0.67±0.12^{bcdefgh}	66.60
2.0 – 3.0	0.73±0.12^{abcdefg}	73.30	2.0 – 3.0	0.47±0.31^{defghı}	46.60	2.0 – 3.0	0.53±0.12^{cdefghı}	66.60
3.0 – 3.0	0.67±0.12^{bcdefgh}	66.60	3.0 – 3.0	0.27±0.12^{ghı}	26.60	3.0 – 3.0	0.80±0.00^{abcdef}	80.00

Recent research found that immature and mature zygotic embryos of einkorn could induce embryogenic callus to a satisfactory level, however, the number of regenerated shoots was less than 0.5 per explant (Özgen et al. 2017) or there was no information regarding the number of regenerated shoots (Yang et al. 2015). Örgeç et al. (2021) found that coleoptile explants

showed the greatest performance for callus induction and indirect plant regeneration compared with root and leaf explants. Furthermore, it was also reported that callus cultures derived from coleoptile explants were capable of plant regeneration whereas callus cultures derived from leaf and root explants were not. A similar result by Sarker & Biswas (2002), stating that root explants of *Triticum aestivum* L. wheat cultivars developed callus that could not regenerate plants, whereas the leaf explants did not produce callus. Benkirane et al. (2000) also showed that the coleoptile explants of *Triticum turgidum* ssp. *durum*. had a higher callus percentage and plant regeneration potential. All these investigations show that the type of explant has a major impact on the regeneration capability. Our findings corroborate previous findings that coleoptile explants were suitable for shoot regeneration and outperformed all other explant types evaluated.

The supporting effect of TDZ on the plant regeneration process has been recently reported for many species (Phippen & Simon 2000; Yucesan et al. 2007; Wang & Bao 2007; Ekmekci & Aasim 2014). Improved regeneration protocols using varied concentrations of thidiazuron (TDZ) applied alone or in conjunction with other plant growth regulators were also developed for a variety of polyploid wheat cultivars (Shan et al. 2000; Ganeshan et al. 2006; She et al. 2013). Miroshnichenko et al. (2016) reported that TDZ enhanced the regeneration capacity of embriyonic callus in cultures of *T. kiharae.* Benlioğlu & Birsin (2017) reported that TDZ had a positive effect on plant regeneration from immature embryo-derived *T. aestivum* L. callus. Different concentrations of TDZ promoted plant regeneration from einkorn callus derived from immature embryo explants (Miroshnichenko et al. 2017). Callus derived from coleoptile explants of *T. monococcum* L. was stimulated by TDZ to regenerate plants (Örgeç et al. 2021). Similarly, in our study, TDZ was a more influential hormone than BA or KIN on the shoot regeneration process (Table 1).

In the second stage of our study, shoots were subcultured on media containing all effective hormonal combinations for 45 days (Table 2). It was noteworthy that 3.66 shoots per explant were induced by the MS medium containing 1 mg L^{-1} TDZ plus 1 mg L^{-1} NAA and 2 mg L^{-1} KIN plus 0.5 mg L^{-1} NAA for 45 days (Figure 2). Induction media comprising 2 mg L^{-1} 2.4-D plus 3 mg L^{-1} BA was found inappropriate for continuous shoot formation. However, the callus formation was observed. This observation is in line with several studies (Dale & Deambrogio 1979; Benkirane et al. 2000; Sarker & Biswas 2002; Alikina et al. 2016). It was found that an increased concentration of 2.4 D induced callusing in *Triticum aestivum* L. (Mahmood et al. 2012) and *T. monococcum* L. (Örgeç et al. 2021). 2,4-D is an auxin-like plant growth regulator that is commonly used in cereals for callus production however it displays low effect for improving somatic embryogenesis and plant regeneration (Miroshnichenko et al. 2017). Similarly, in our study, interaction with 2.4 D increased the callusing potential while decreasing shoot formation capacity. The variability in callus formation frequency in return for various levels of 2.4-D may be due to differences in genes controlling callusing or genes may not express themselves fully in some cultivars contrary to others supplemented with an optimum concentration of 2.4-D. Our findings are in concurrence with other researchers who also suggested genotypic differences of wheat for callus formation and regeneration abilities (Kilinc 2004; Nasircilar et al. 2006; Hassan et al. 2009; Örgeç et al. 2021). Although various researchers had standardized the concentration of 2.4-D at the optimum level for diverse genotypes of wheat (Satyavathi et al. 2004; Sarker & Biswas 2002) the induction media should be standardized for maximum callusing in einkorn wheat.

Table 2- Shoot regeneration obtained from *in vitro*-grown regenerants subcultured on MS medium containing the most effective hormone combination. Mean values (± SD) with the different letters in the same columns are significantly different (P < 0.05)

Combinations of PGRs	*Mean number of shoots/explant*	*Shoot formation percentage (%)*
Control	$0.66^{ab} \pm 0.20$	26.66
0.5 mg L^{-1} TDZ	$2.33^{a} \pm 0.88$	73.33
1 mg L^{-1} TDZ+1 mg L^{-1} NAA	$3.66^{a} \pm 0.66$	73.33
2 mg L^{-1} TDZ	$3.00^{a} \pm 0.33$	46.66
0.5 mg L^{-1} BA+2 mg L^{-1} NAA	$2.66^{a} \pm 0.57$	60.00
3 mg L^{-1} BA+0.5 mg L^{-1} IAA	$3.33^{a} \pm 0.88$	66.60
3 mg L^{-1} BA+2 mg L^{-1} 2.4-D	$0.46^{ab} \pm 0.33$	60.00
0.5 mg L^{-1} KIN+1 mg L^{-1} 2.4-D	$3.00^{a} \pm 0.57$	33.33
1 mg L^{-1} KIN	$3.00^{a} \pm 1.00$	60.00
2 mg L^{-1} KIN+0.5 mg L^{-1} NAA	$3.66^{a} \pm 0.33$	60.00

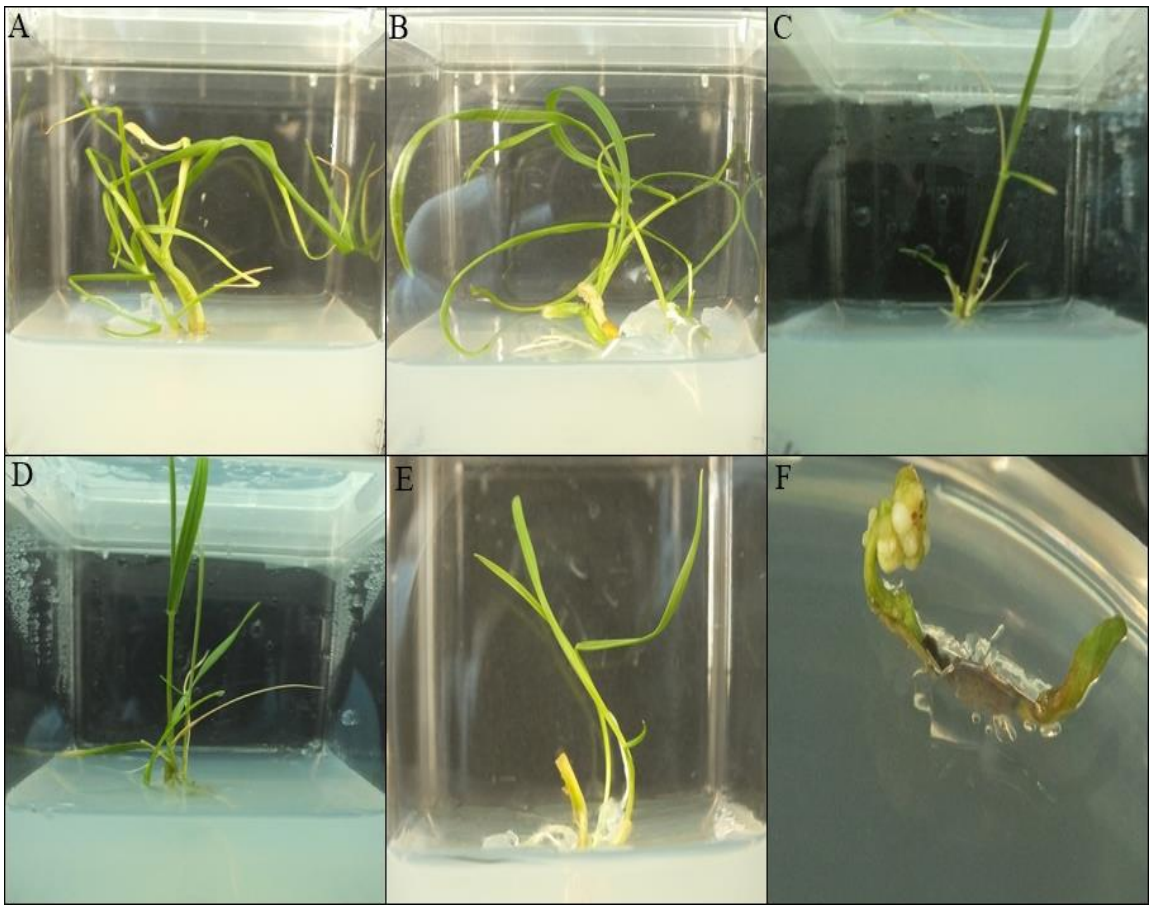

Figure 2- The shoots were subcultured on the MS medium containing the most effective hormone combinations. (A) 2.0 mg L^{-1} KIN + 0.5 mg L^{-1} NAA (B) 1.0 mg L^{-1} TDZ +1.0 mg L^{-1} NAA. (C) 0.5 mg L^{-1} TDZ. (D) 0.5 mg L^{-1} TDZ + 2.0 mg L^{-1} NAA. (E) 1.0 mg L^{-1} KIN. (F) 3.0 mg L^{-1} BA + 2.0 mg L^{-1} 2.4-D

Early in the 1930s, indole-3-acetic acid (IAA) was identified to be the most effective auxin in promoting the development of adventitious root (Thimann & Koepfli 1935), and since then IAA has been widely used to induce adventitious root formation in the clonal propagation of various species (Shu et al. 2019). Although only a few studies have focused on the root formation for *in vitro* study of wheat, IAA has been one of the most widely used auxin-hormone to induce root induction (Kopertekh & Stribnaya 2003; Örgeç et al. 2021).

In our study, to induce root formation, regenerated shoots (45 days of culture) were cultured on MS medium with different concentrations of IAA ranging from 0.5 to 5 mg L^{-1} (Table 2). They formed roots in one week. Among the different IAA concentrations tested, 2 mg L^{-1} IAA was the most effective hormone concentration for rooting (Figure 3). 12.33±0.88 roots produced per regenerated shoot. When IAA concentration was increased from 0.5 to 2 mg L^{-1}, more root formation was observed. However, when IAA concentration was increased from 2 to 5 mg L^{-1}, the mean number of roots began to decline (Table 3). To get the acquisition of the meristematic competence of the cells, auxin is accepted as the most effective hormone in tissue culture experiments. However, it was known that after this competence was established, excessive auxin concentration inhibited further adventitious or embryonic root development (Gurel & Wren 1995; Charriere et al. 1999).

Table 3- Effects of the tested auxins on rooting. Mean values (± SD) with the different letters in the same columns are significantly different (P < 0.05)

Auxin concentration	*Mean number of roots/shoot*	*Root formation percentage (%)*
0.5 mg L^{-1} IAA	$9.00^{b} \pm 1.00$	100
1 mg L^{-1} IAA	$11.67^{a} \pm 0.66$	100
2 mg L^{-1} IAA	$12.33^{a} \pm 0.88$	100
3 mg L^{-1} IAA	$7.33^{bc} \pm 0.33$	100
5 mg L^{-1} IAA	$5.33^{c} \pm 0.88$	100

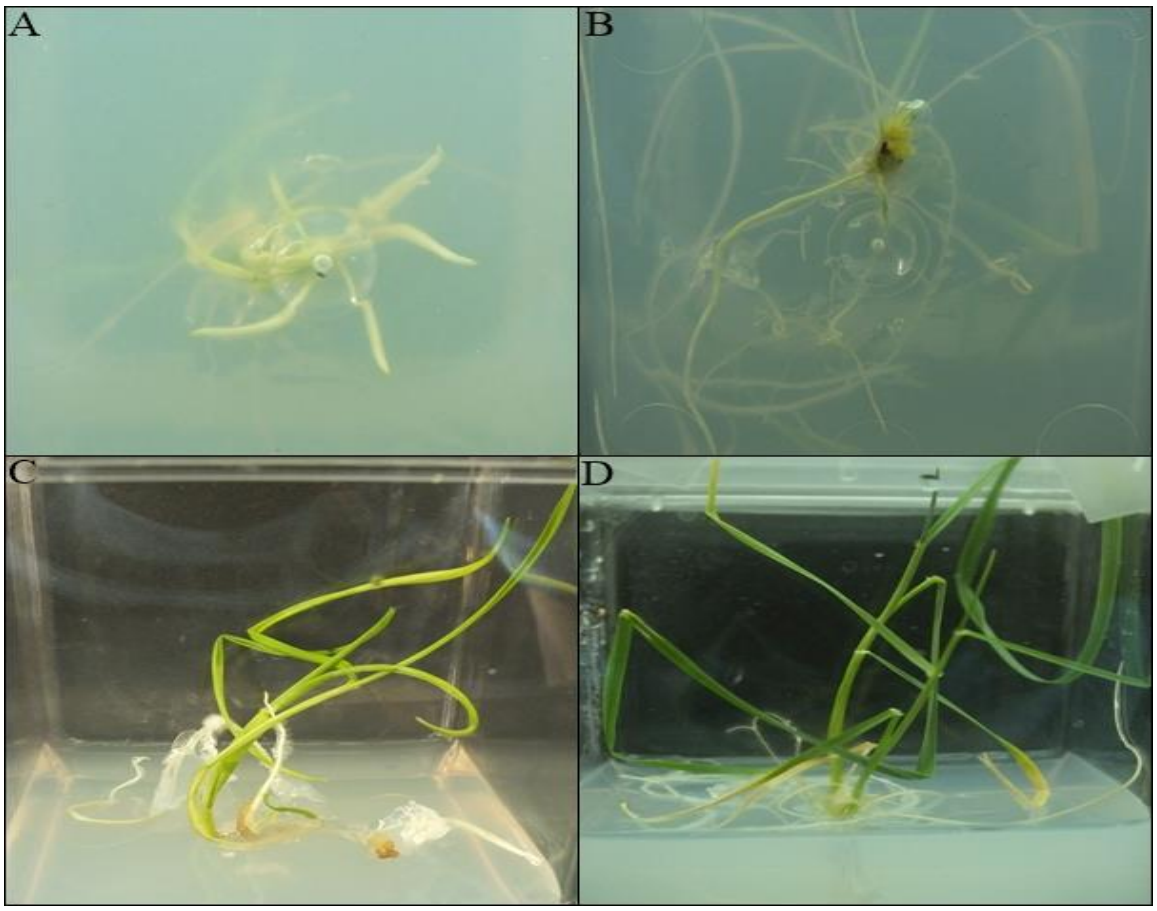

Figure 3- Effect of 2 mg L^{-1} IAA on root formation from regenerated shoots

Finally, these well-developed plantlets were transferred to plastic pots containing a mixture of vermiculite and soil (1:2). For acclimatization, they were kept in the climate room for one week. After one week, the plantlets were transferred to pots containing commercial soil, kept under room conditions (Figure 4. a–f). Eventually, all the plantlets were established in the field, with 100% survival.

Figure 4- Direct shoot and plant regeneration from coleoptile explants of *Triticum monococcum* L. (A) Rooting of the regenerated shoots on medium containing 2.0 mg l^{-1} IAA after three weeks in culture. (B-C) Regenerated plants transferred to pots containing a mixture of vermiculite and soil (1:2) under climate room conditions. (D-F) Regenerated plants transferred to pots containing sterile soil under room conditions.

4. Conclusions

This article describes an efficient protocol for direct plant regeneration using somatic tissues in einkorn by testing different concentrations of TDZ, KIN, and BAP in combination with NAA, IAA, and 2.4-D. The present investigation elucidates that the coleoptile part of wheat can serve as a potential source for direct shoot regeneration under suitable conditions. This method is an efficient one for the *ex vitro* conservation of kinds of ancient wheat genetic resources and clonal propagation.

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