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Effects of different growth regulators on regeneration of Turkish upland rice

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ABSTRACT

Rice is a staple crop grown in the world especially in the Asian region. The rice faces with different biotics and abiotic stress factors, especially drought stress. Turkish upland rice is one of the special rice types grown on drought stress condition, having a large potential value. Our aim was to analyse the effects of different concentrations of BAP+NAA and KIN+2,4-D combination on rice grown *in vitro* condition. For this purpose, mature seeds of Turkish upland rice were selected as starting material for direct/indirect regeneration response in serial treatment. The best medium for induction of shoot regeneration was MS<sub>1</sub> supplemented with 1.5 mg L<sup>-1</sup> BAP and 0.5 NAA mg L<sup>-1</sup>. The results showed that the maximum callus formation (65.54 %) was obtained on MS medium containing 2.4-D (2.0 mg L<sup>-1</sup>) within 21 days from nodes. With this study, we have developed a plant tissue culture for Turkish upland rice, suggesting positive methods for rice biotechnology.

Keywords:

Callus  
Plant Regeneration  
BAP  
NAA  
KIN  
2.4-D

Farklı büyüme düzenleyicilerin Türk kır çeltiği rejenerasyonuna etkisi

ÖZET

Çeltik bitkisi, tüm Dünya’da ve özellikle Asya kıtasında yetişen temel bir üründür. Çeltik, kuraklık stresi gibi farklı abiyotik ve biyotik stres faktörleri ile karşı karşıyadır. Türk kır çeltiği, kuraklık stres koşullarında yetişen ve büyük bir potansiyele sahip olan üstün çeltik türlerinden biridir. Bu çalışmanın amacı, BAP + NAA ve KIN + 2.4-D kombinasyonunun farklı konsantrasyonlardaki *in vitro* koşullarda yetiştirilen çeltik üzerindeki etkilerini incelemektir. Bu amaçla, başlangıç materyali olarak olgun çeltik tohumları kullanılmış olup direkt ve indirekt rejenerasyon sonuçları kaydedilmiştir. Gövde rejenerasyonu indüksiyonu için en iyi ortam, 1.5 mg L<sup>-1</sup> BAP ve 0.5 NAA mg L<sup>-1</sup> ile desteklenmiş MS<sub>1</sub> olmuştur. Sonuç olarak, gövde nodların 21 gün içinde 2.4-D (2.0 mg L<sup>-1</sup>) içeren MS ortamı üzerinde maksimum kallus oluşumunun (% 65.54) elde edildiğini kaydedilmiştir. Bu çalışma ile Türk kır çeltiği için doku kültürü optimizasyonu geliştirmiştir ve çeltik biyoteknolojisi açısından pozitif metotlar sunulmuştur.

Anahtar Sözcükler:  
Kallus  
Bitki rejenerasyonu  
BAP  
NAA  
KIN  
2.4-D

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1. Introduction

Cultivated rice is world’ single most important grain (Horuz, 2014). Rice is stable food for more than half of the Asian consumption. Upland rice, a dry farming ecotype of cultivated rice, is commonly directly seeded without a water layer cover. Additionally, upland rice is one of special rice types grown on limited irrigation conditions (Din et al., 2016; Kaya et al., 2017). The rice is proper for low-lying, hilly countryside, rainy mountainous areas and waterlogged upland regions, and rice paddy fields with limited water irrigation (Jahan et al., 2017).

Furthermore, the upland rice is more drought

resistant than lowland rice. In addition, Jahan et al., (2015) have been highlighted to identify the genetic and biochemical mechanisms helping rice to overcome with toxicities of toxic substance like iron ions. Thus, given a global shortage of water resources, the drought resistant properties of upland rice make it ideal for sustainable rice production and food security. Abiotic stress such as drought stress is an important issue for rice production around the world due to lower productivity as compared with flooded rice. Furthermore, 12 % of global paddy production is upland rice. The increases in world rice production over the past years has been resulted from successes in research and the transfer of biotechnology. However, these successes had virtually much effect on

upland rice production in the world (Karakütük, 2017). Also, recently years, new upland rice cultivars have been developed on around the world (Din et al., 2016). Some of the cultivars show high drought tolerance (Yang et al., 2004) and high yield potential (Bouman et al., 2006).

Upland rice improvement, the applications of biotechnological methods are limited by the availability of *in vitro* plant regeneration approaches (Din et al., 2016). On the other hand, plant tissue culture is one the most important steps of plant regeneration and gene transfer among modern methods (Kaya et al., 2013). In addition, several strategies need to be devised to improve upland rice productivity via the conventional breeding methods along with recent achievement in biotechnological breeding to balance the world consumption of rice (Shahsavari, 2010; Shahsavari et al., 2010). For the reason, upland rice genetic transformation needs to be applied to address abiotic factors such as drought stress, hindering the upland rice production. The upland rice genetic transformation systems require an essential step of *in vitro* plant regeneration from explant of upland rice.

Until now, several reports have been shown *in vitro* regeneration of upland rice especially Malaysian upland rice (Din et al., 2016; Zhao et al., 2011; Shahsavari, 2011; Shahsavari et al., 2010; Shahsavari, 2010; Geng et al., 2008). To the best of our knowledge, no reports are available in literature for tissue culture of Turkish upland rice. Therefore, the purpose of this research constitutes the first ever successful and complete *in vitro* propagation of protocol for Turkish upland rice.

## 2. Materials and Methods

### 2.1. Source of explants

Fresh and healthy mature seeds were collected from Eastern Anatolia region of Turkey in 2015. Turkish upland rice local namely Kır Çeltiği were used for this research. Seeds were stored in plastic bags at 4°C as the source of explant for all experiments.

### 2.2. Surface sterilization

Mature seeds of Turkish upland rice were dehusked and rinsed thrice using sterile double distilled water. The seed were then sterilised by immersed in 60 % ethanol for 55 seconds, then washed 2 times with double distilled water, followed by 50 % Clorox for 18 minutes. Treated seeds were washed again with double distilled water for five times. Treated seeds were blotted dry on sterile tissue paper. The sterile seeds were placed on MS media and kept at 25±2 °C.

### 2.3. Indirect and direct regeneration from explants

Sterilized seeds were cultured on a basal MS (Murashige and Skoog, 1962) medium, including 30 g

L<sup>-1</sup> sucrose and 0.3 % phytigel was designed as MS<sub>1</sub>. For subculture, all medium were transferred into fresh same media in same condition after 15 days. Plant regeneration from seed grown well. After 25 days, we used the plants for source of explants. For direct regeneration, nodes and leaves were cultures on medium consisting MS<sub>1</sub>, BAP (0.5, 1, 1.5, 2.0 mg L<sup>-1</sup>) alone and in combination with NAA (0.5, 1.0 mg L<sup>-1</sup>). Also explants were places on MS<sub>1</sub> supplemented with 2,4-D alone (0.5, 1, 1.5, 2.0 mg L<sup>-1</sup>) and in combination with KIN (0.5, 1.0 mg L<sup>-1</sup>). Each treatment contained 12 seeds with 3 replicates and even the experiments were repeated 3 times. The sterilized seeds were incubated under 16/8 h (light/dark) photoperiod at 25±2 °C for 25 days.

### 2.4. Statistical analysis

The experiments were arranged in a completely randomized design with three replications. All reactions were performed with three technical and three biological replicates. One-way analysis of variance was used on experimental data in addition to post hoc Tukey's test has via IBM SPSS for windows v.20.

## 3. Results and Discussion

This is the first complete protocol for *in vitro* tissue culture optimization of Turkish upland rice. *In vitro* seeds germination and seedling development of Turkish upland rice were successfully performed (Fig. 1). Multiple shoot and root were achieved explant of Turkish upland rice onto the MS<sub>1</sub> medium. After incubation on the MS media, seeds became swollen quickly and germination occurred within the first 5 days of culture (Fig. 1. A-C) and the growth of regeneration was started followed root (Fig. 1. D). After 15 days, (Fig. 1. E) the samples were subcultured (Fig. 1. F).

Leaves and nodes from aseptically grown plants of Turkish upland rice (Fig. 1. G) were used as explants. The leaves (Fig. 1. H) and the nodes (Fig. 1. I) were excised from 25 days-old seedlings, and were cultured on MS medium containing various concentration of BAP+NAA and KIN+2,4-D. *In vitro* tissue culture continued over a period of four weeks to the increase stock. Node explants with leaf primordia appeared seven days after culture initiation from node explants (F1. J). Adventitious shoot or multiple shoots (Fig. 1. J) formation was achieved directly from the cut edges of the node explants. There was no regeneration from leaf explant (data not shown) (Fig. 1. J-K).

The mean shoot number per explant varied between 8.90±1.55 and 30.34±0.59, and the highest shoot formation was 91.17 % on MS medium containing 1.5 mg L<sup>-1</sup> BAP + 0.5 mg L<sup>-1</sup> NAA (Table 1). Also, the lowest frequency of shoot formation from node explants was 41.46 % recorded MS medium supplemented with 0.5 mg L<sup>-1</sup> BAP alone (Table 1).

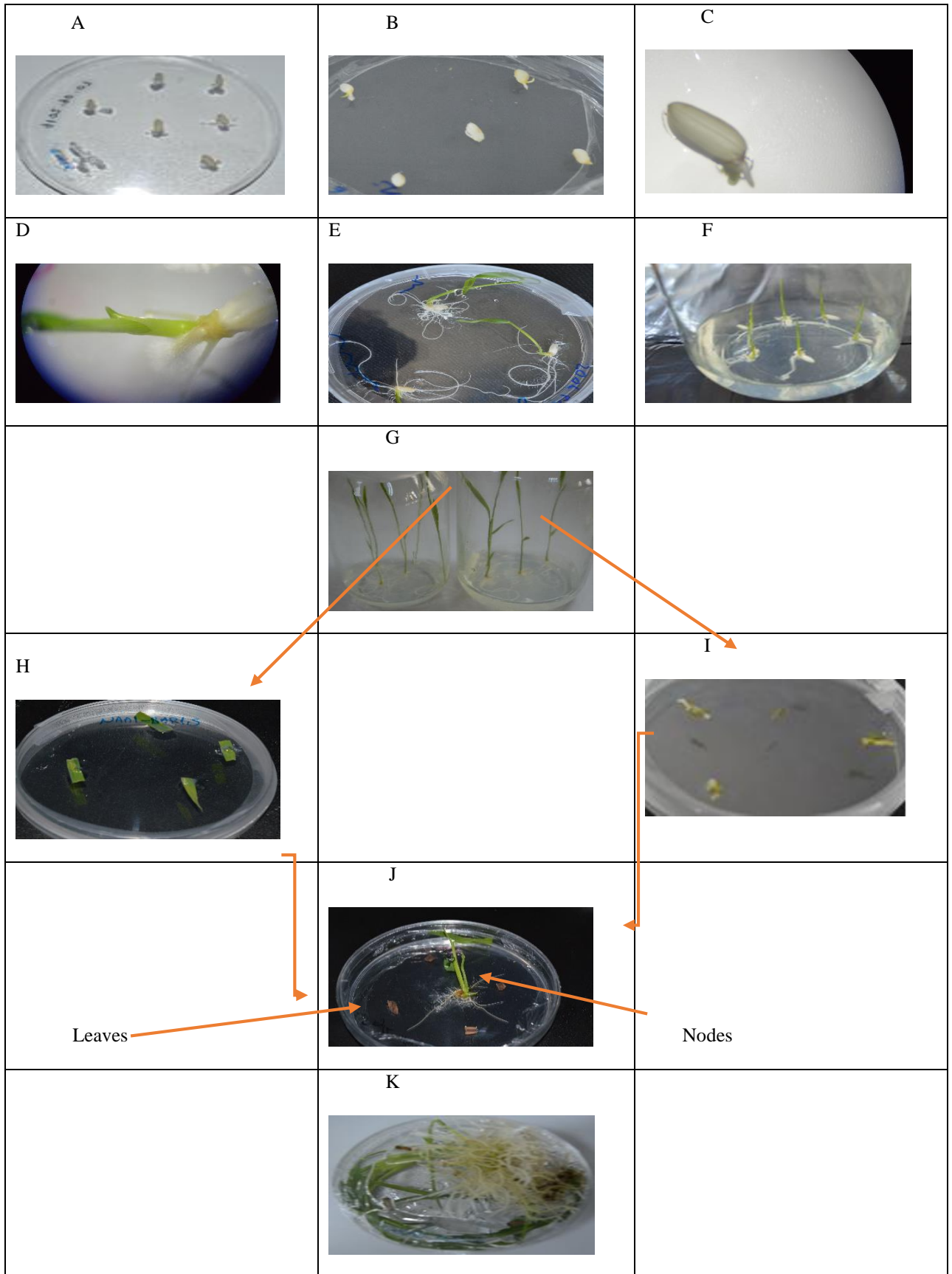


Figure 1. Direct plant regeneration from seed on MS<sub>1</sub> medium

Among different concentrations of 2,4-D and KIN tested, MS<sub>1</sub> media containing 0.5 mg L<sup>-1</sup> 2,4-D and 1.0 mg L<sup>-1</sup> KIN produced the highest shoot formation (75.82 %) with healthy regeneration. The lowest shoot formation (43.37 %) was achieved on the MS<sub>1</sub> consisting of 2.0 mg L<sup>-1</sup> 2,4-D alone (Table 2). The mean shoot number per explant varied between

08.21±0.97 and 18.70±1.18. The highest regeneration number was 18.70±1.18 on MS medium containing 1.0 2,4-D (mg L<sup>-1</sup>) + 1.0 KIN (mg L<sup>-1</sup>) (Table 2). Also, the mean highest callus induction was 65.54 % in MS medium supplemented with, 2.0 mg L<sup>-1</sup> 2,4-D alone (Table 2).

Table 1. The effects of different BAP and NAA hormone concentrations on node explant

MS Medium	Plant Growth Regulators		The shoot formation (%)	The mean shoot number per explant
	BAP (mg L <sup>-1</sup> )	NAA (mg L <sup>-1</sup> )		
1	0.5	-	41.46 <sup>l</sup>	12.10±1.30 <sup>b</sup>
2	1.0	-	50.44 <sup>k</sup>	09.89±1.95 <sup>f</sup>
3	1.5	-	64.66 <sup>h</sup>	15.33±0.88 <sup>e</sup>
4	2.0	-	73.07 <sup>f</sup>	19.02±0.77 <sup>d</sup>
5	0.5	0.5	61.55 <sup>i</sup>	06.20±0.90 <sup>g</sup>
6	1.0	0.5	81.36 <sup>c</sup>	21.51±1.52 <sup>b</sup>
7	1.5	0.5	91.17 <sup>a</sup>	30.34±0.59 <sup>a</sup>
8	2.0	0.5	85.22 <sup>b</sup>	25.13±2.02 <sup>b</sup>
9	0.5	1.0	67.84 <sup>g</sup>	18.63±1.10 <sup>d</sup>
10	1.0	1.0	78.42 <sup>d</sup>	21.80±1.80 <sup>c</sup>
11	1.5	1.0	75.66 <sup>c</sup>	20.09±2.39 <sup>d</sup>
12	2.0	1.0	57.63 <sup>j</sup>	08.90±1.55 <sup>f</sup>

Table 2. The effects of different 2,4-D and KIN hormone concentrations on node explant

MS Medium	Plant Growth Regulators		The mean callus formation (%)	The shoot formation (%)	The mean shoot number per explant
	2,4-D(mg L <sup>-1</sup> )	KIN(mg L <sup>-1</sup> )			
1	0.5	-	37.95 <sup>d</sup>	69.15 <sup>b</sup>	15.12±1.71 <sup>d</sup>
2	1.0	-	45.52 <sup>c</sup>	69.20 <sup>d</sup>	13.13±1.19 <sup>c</sup>
3	1.5	-	53.46 <sup>b</sup>	50.46 <sup>j</sup>	15.33±0.88 <sup>e</sup>
4	2.0	-	65.54 <sup>a</sup>	43.37 <sup>k</sup>	08.21±0.97 <sup>j</sup>
5	0.5	0.5	7.20 <sup>j</sup>	65.04 <sup>f</sup>	10.90±0.90 <sup>f</sup>
6	1.0	0.5	10.22 <sup>i</sup>	58.90 <sup>g</sup>	10.01±1.72 <sup>g</sup>
7	1.5	0.5	33.43 <sup>e</sup>	56.34 <sup>i</sup>	09.34±0.90 <sup>h</sup>
8	2.0	0.5	24.43 <sup>g</sup>	58.39 <sup>h</sup>	10.90±2.01 <sup>g</sup>
9	0.5	1.0	26.72 <sup>l</sup>	75.82 <sup>a</sup>	17.63±2.11 <sup>b</sup>
10	1.0	1.0	20.38 <sup>h</sup>	71.80 <sup>b</sup>	18.70±1.18 <sup>a</sup>
11	1.5	1.0	35.38 <sup>d</sup>	70.93 <sup>b</sup>	16.19±1.36 <sup>d</sup>
12	2.0	1.0	3.88 <sup>k</sup>	66.39 <sup>c</sup>	14.44±1.15 <sup>c</sup>

In this research, the tissue culture optimization of Turkish upland rice was evaluated. The finding obtained from research have emphasised the best concentration and composition of plant growth regulators (PGRs) (2,4-D, BAP, KIN and NAA). Variety concentration of 2,4-D growth regulator is one of the most effective ways for acquiring callus formation (Karthikeyan et al., 2009). In addition, BAP, NAA, NAA and KIN growth regulator combinations are one of the most effective combinations for regeneration of plants (Ali et al., 2004; Kurt et al., 2008; Shahsavari, 2010; Sahoo et al., 2011; Din et al., 2016;). Until now, there are several reports in

Malaysian upland rice and Chinese upland rice such as those reported by Ali et al., 2004; Shahsavari, 2010; Din et al., 2016). However, there is no study on tissue culture optimization of Turkish upland rice.

Further, the use of 2,4-D was observed as for micropropagation through calluses for our research. However, our finding proved that the 2,4-D growth regulator 2 mg L<sup>-1</sup> alone induced better callus induction frequency (65.54%). Our finding was also in contrast to previous findings on other upland rice such as Malaysian upland rice on grown MSB<sub>5</sub> and MS medium consist of 2,4-D only (Shahvari, 2010; Din et al., 2016).

Nevertheless, our results were agreements with Sah et al., (2014) who observed that 2.4-D growth regulator with variety of concentration gave better response to callus formation in *Oryza sativa indica*. Also, our observations suggested that high concentration of 2.4-D ( $2.0 \text{ mg L}^{-1}$ ) caused the callus become brown and low quality for *in vitro* plant regeneration (Data not shown). Ali et al. (2004) and Rueb et al. (1994) suggested high does concentration of 2.4-D could induce a negative effect on callusing. Similar reduction in quantity of 2.4-D was reported Malaysian upland rice (Din et al., 2016). As a result of our research, we suggested that less than  $2 \text{ mg L}^{-1}$  2.4-D alone resulted in the highest callus formation.

Direct plant regeneration from seeds was observed within 7 days culture Seed germination often preceded regeneration on MS media containing the variety concentration of BAP. Also, the average of plant direct regeneration from seed on MS medium added with BAP ( $0.5, 1, 1.5, 2 \text{ mg L}^{-1}$ ) were statistically different from each other. In contrast, Wang et al., (2011) have studied hybrid of indica rice species for tissue culture system, and the growth regulator concentrations (BAP, NAA) were attempted for *in vitro* plant regeneration from seed. We applied the same growth regulators, but their results were not similar to ours. Therefore, the results of that study were in contrast with our findings. Because our genotypes and type of rice are different from that study.

In the current research, Turkish upland rice was used for tissue culture optimization through explants from leaves and nodes. Nodes explant in monocot plants is an excellent source of analysing the ability of tissue culture responsive to initiate regeneration plants for rice (Ramesh et al., 2009; Kurt, 2015, Kurt, 2016, Karakütük, 2017).

The effect of BAP+NAA was studied and recorded that the growth regulator concentration enhanced the regeneration of plant from explant for nodes (Kurt, 2016). Similarly, in some Turkish varieties of rice, multiple plant regeneration was achieved in added of MS medium supplemented BAP+NAA. Simultaneously, positive effect of NAA in combination with BAP has been previously reported to facilitate plant regeneration in rice (Kurt, 2008; Shekar et al., 2016; Kurt, 2016). The observed results revealed that KIN was an effective operation in plant regeneration of rice culture (Shekar, 2016; Kurt, 2016). However, our research recorded that the combination 2.4-D and KIN negative effected for plant regeneration from explant of nodes. Based on our findings, we suggested that a combination of BAP and NAA produced higher percentage of Turkish upland rice regeneration from explant of nodes. Also, our results indicated that a combination of 2,4-D and KIN produced less percentage of Turkish upland rice regeneration from explant of nodes. Since the same growth regulator composition is not suitable for all rice variety, the modifying media were diversified to overcome the genotypic influence for particular rice

varieties either upland rice or wetland rice.

#### 4. Conclusion

The current research showed establishment on callus induction using 2.4-D for Turkish upland rice from seed. Also, our results indicated that plant regeneration from seed using BAP, NAA, 2.4-D and KIN for Turkish Upland rice. Also, it was recorded that best concentrations of growth regulators alone and in combinations (NAA+BAP and 2.4-D+KIN) has significantly enhanced the regeneration of Turkish upland rice from explant of nodes.

In conclusion, an efficient technique for *in vitro* germination of seeds and regeneration of callus induction of seed from seed and explant for Turkish upland rice has been described. This is the first report about *in vitro* propagation of Turkish upland rice. Our findings could be useful for tissue culture optimization of Turkish upland rice.

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