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Research Article

Effect of picloram and 2,4-D on plant regeneration from mature and immature embryos of moroccan durum wheat varieties

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Abstract An efficient genetic transformation protocol is a fundamental requirement for high regeneration capacity from cultivated durum wheat (*Triticum durum*) varieties. In this study, wereportedtheeffectsoftwoauxins,2,4-dichlorophenoxyaceticacid(2,4-D)and4-amino-3,5,6-trichloropicolinicacid(picloram), at a concentration of 2 mg/Laloneandincombination on the embryogenic callus and plantlet regeneration of four durum wheat varieties (Amria, Chaoui, Marouane, and Tomouh) using mature embryos (MEs) and immature embryos (ImEs). Significanteffectsofvariety,culturemedium(theauxinused),andvariety-mediuminteraction were observed on the callus weight and plantlet regeneration of both MR and ImE explants. The medium used for callus induction significantly affected plantlet regeneration ($p < 0.001$). Comparedto2,4-D, picloram led to a higher plantlet regeneration rate in both ME and ImE explants (19.8% and 40.86%, respectively). Plantlet regeneration also varied significantly depending on the variety and medium used. PicloramledtohighplantletregenerationofbothME and ImE explants in all varieties except Tomouh, which showed high plantlet regeneration of ME explants in 2,4-D. A comparison of ME and ImE responses indicated that ImEs are the best explants for high plantlet regeneration in durum wheat. Ourfindingssuggestthatpicloramisthebestauxin and should be used instead of 2,4-D due to its positive effect on increasing plant regeneration of durum wheat ME and ImE explants.

Keywords 2,4-D, picloram, plantlet regeneration, somatic embryogenesis, *Triticum durum*

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Introduction

Durum wheat (*Triticum durum* L.) is the most important cereal crop in the Mediterranean basin. In Morocco, durum wheat is cultivated over an area of 890 hectare from four million used for cereal crops (USDA 2019). The country's wheat productivity has been affected by various biotic stresses (insect, root rot, nematodes, and viruses) and abiotic stresses (drought) (Karrou 2003). The most significant environmental stress that affect durum wheat crops is drought, it causes a severe decrease in performance. Genetic transformation provides a method for genetic manipulation of wheat in order to have resistance to biotic and abiotic stresses. Genetic improvement of wheat depends mostly on the ability of the plant tissue to regenerate into whole plants (Ren et al. 2010). Regeneration of durum wheat plants *in vitro* is a crucial requirement for genetic improvement through genetic transformation (Takumi and Shimada 1997).

The induction of high-quality embryogenic calli is an essential step for the plant regeneration from mature and immature embryo culture of wheat. Nowadays numerous studies have been conducted in order to improve embryogenic callus production. Wheat response to callus induction and plant regeneration is also influenced by several factors, such as genotype (Mahmood and Razzaq 2017; Wang et al. 2018) and environmental conditions, such as

media components (Mahmood and Razzaq 2017) and incubation conditions (Fennell et al. 1996). Plant hormones, particularly auxins, have been demonstrated to be one of the most important factors for the induction of somatic embryogenesis (Barro et al. 1998; Wu et al. 2009). In the *in vitro* culture of calli, exogenous application of a high concentration of auxins was effective for plant regeneration (Jiménez 2005; Karami and Saidi 2010). The importance of plant growth regulators has been demonstrated for a large number of cereals, especially the levels of synthetic auxins for callus induction and plant regeneration. 2,4-D is considered to be the main auxin used for induction of somatic embryogenesis in wheat (Miroshnichenko et al. 2009), barely (Zapata et al. 2004) and rice (Ming et al. 2019). Other substances with auxin-like activities such as dicamba (Miroshnichenko et al. 2016; Ren et al. 2010) and picloram (Ahmadpour et al. 2018; He and Lazzeri 2001; Miroshnichenko et al. 2016) have been used as alternatives. The general mechanism by which Auxin works is by promoting the degradation of transcriptional regulators known as Aux/IAA proteins. Aux/IAA degradation requires the F-box-containing Transport Inhibitor Resistant1/Auxin Signaling F-Box (TIR1/AFB) proteins. The TIR1/AFB auxin receptor family comprises TIR1 and AFB1 to -5, which all have been shown to function as auxin receptors. The picolinate-type auxin analogs are selectively recognized by AFB4/5−Aux/IAA co-receptors due to the presence of an extension on the N-terminal end of the protein and bind to the synthetic auxin picloram, unlike the other members of the family reviewed by (Ma et al. 2018). However, 2,4-D appear to act primarily via TIR1 and the homologous AFB1, AFB2, and AFB3 (Dharmasiri et al. 2005)

For *in vitro* regeneration of wheat plants, many tissues have been used, immature embryos (Aadel et al. 2016; Wang et al. 2014), mature embryos (Ekom et al. 2013; Senhaji et al. 2021), leaf segments (Yu et al. 2012), coleoptiles (Benkirane et al. 2000), inflorescences and scutellum (He and Lazzeri 2001; Yadav et al. 2020) and also from anthers (Redha and Suleman 2011). These tissues vary in their ability to regenerate whole plants; however, immature embryos are regarded as among the most suitable explants (Jones 2005; Yang et al. 2015). Very little information is available on tissue culture of the tetraploid durum wheat (*Triticum durum*) which is the most recalcitrant species among wheats. Therefore, we used two auxins, picloram (2 mg/l), 2,4-D (2 mg/l) and the combination of picloram and 2,4-D for improving the callus induction and regeneration of immature embryos. Further, to overcome the luck of availability of immature embryos during year, we employed same experiments on mature embryos. In both, immature and mature embryos we used four Moroccan varieties; 'Amria', 'Chaoui', 'Marouane' and 'Tomouh'.

Materials and methods

Plant material and preparation of explants

Four Moroccan durum wheat varieties, 'Amria', 'Chaoui', 'Marouane', and 'Tomouh', was used as different genotypes in this study. The seeds were procured from the Experimental Research Station of INRA, Marchouch, Rabat, Morocco. Immature and mature embryos were used as explant to induce callogenesis. The plants were grown in a greenhouse and the seeds were collected from the milky phase, after 12 to 16 days post-anthesis. The immature and mature seeds were sterilized by ethanol 70% (v/v) for 3 minutes, and then soaked in a 2.4% sodium hypochlorite and tween 20 solution for 15 minutes (immature seeds) or 30 minutes (mature seeds) with agitation. Then, they were rinsed three times in sterile distilled water (under laminar flow). The mature seeds were soaked in sterile distilled water for overnight at room temperature to facilitate the embryo excision. The mature and immature embryos were aseptically excised and placed on a culture medium with the embryo-axis in contact with the medium.

Culture medium, induction and regeneration

Mature and immature embryo explants were transferred to four different media for callus formation and maintenance. The 4 tested media basically contained MS salts (Murashige and Skoog 1962) supplemented by 100 mg/l myo-inositol, 150 mg/l asparagine, 20 g/l saccharose, and the first callus induction medium (M1) contains 2 mg/l 2,4-D, the second medium (M2) contains 2 mg/l picloram, the third medium (M3) contains 2 mg/l of each picloram and 2,4-D. The fourth medium (M4) was auxin-free. All media used in this study were solidified with 2.5 g/l phytagel, the pH was adjusted to 5.7, and they were autoclaved at 121°C for 20 minutes. The cultures were incubated in the dark at 25°C and subcultured two times onto a fresh medium at 15 days intervals. After 40 days, the callus weight was recorded.

For differentiation of callus into shoots, after five weeks calli initiated from mature and immature embryos were transferred to the regeneration medium (Iraqi et al. 2005) and incubated in the light (16 h per day) and at of 25°C.

The regeneration rate was calculated after transfer of the callus. Percentage of plants regeneration (PR) and number of plantlets regenerating per callus (NPR/Cal) was calculated as follows:

- Plant regeneration percentage = (number of calli regenerated/number of calli transferred to regeneration medium) × 100 (Tang et al. 2006);
- Number of plantlets regenerating per callus (NPR/Cal) = (number of plantlets regenerated/total number of callus);
- Number of plantlets regenerated per regenerating callus (NPR/RCal) = (number of plantlets regenerated / total of regenerating callus).

Experimental design and statistical analysis

A randomized complete block design (RCBD) was used with 4 varieties and 4 media (4 × 4 = 16 treatments). The treatments consisted of 5 replications of each medium for each variety; each dish contained 20 explants. Analysis of Variance (ANOVA) was performed to analyze the different parameters taken: weight of callus and percentage of plant regeneration, using the General Linear Model (GLM) procedure in SAS (SAS Institute 1985). Mean of treatments was compared using Duncan's Multiple Range test (d Steel and Torrie 1986).

Results

Callus initiation and growth

In the present study, four different media were tested to determine the best medium for successful callus induction and plant regeneration (Fig. 1). The percentage of callus induction was 100% for mature and immature embryos from the four varieties used in the first three media (Table 2), except for M4 media without auxin, in which only roots were developed.

Callus weight was influenced not only by variety or medium but also by the combination of variety ⁎ medium interaction (Table 1). Among the four varieties tested, 'Marouane' showed the best mean for mature embryos callus weight (2.78 g) after 4 weeks of incubation in different media, followed by 'Amria' (2.20 g), 'Tomouh' (2.20 g) and 'Chaoui' (2.06 g) (Table 2). For immature embryos, 'Marouane' (3.73 g) showed the best mean callus weight after 4 weeks of incubation in different media, followed by 'Chaoui' (3.09 g), 'Amria' (3.02 g) and 'Tomouh' (2.50 g) (Table 2). On the other hand, using 2,4-D (M1) as an auxin revealed a best growth rate of mature embryos callus weight (2.81 g) followed by MS medium supplemented with the combination of 2,4-D and picloram (M3) (2.73 g) and picloram (M2) (1.37 g) (Table 2). However, for immature embryos callus weight, the best growth rate was observed using the combination of 2,4-D and picloram media (4.07 g) followed by 2,4-D (3.06 g) and picloram (2.12 g) (Table 1). On all media, all genotypes showed good callus growth at different levels, the rate of increase in callus weight was proportional to the incubation period

Plantlet regeneration

After 4 weeks of callus induction, they were transferred to the regeneration media. Then, the plantlets regeneration was recorded (Table 2). The Induction and maintenance media used for callus induction influenced plantlet regeneration significantly ($P < 0.001$) (Table 1). Using mature embryos, picloram (19.80%) showed the highest plantlet regeneration rate followed by 2,4-D (13.66%) and the combination of 2,4-D of picloram (3.09%). For immature embryos, picloram (40.86%) showed the highest plantlet regeneration, followed by 2,4-D (31.96%) and the 2,4-D, picloram combination (10.59%). Even though, the use of 2,4-D as auxin and 2,4-D, picloram combination

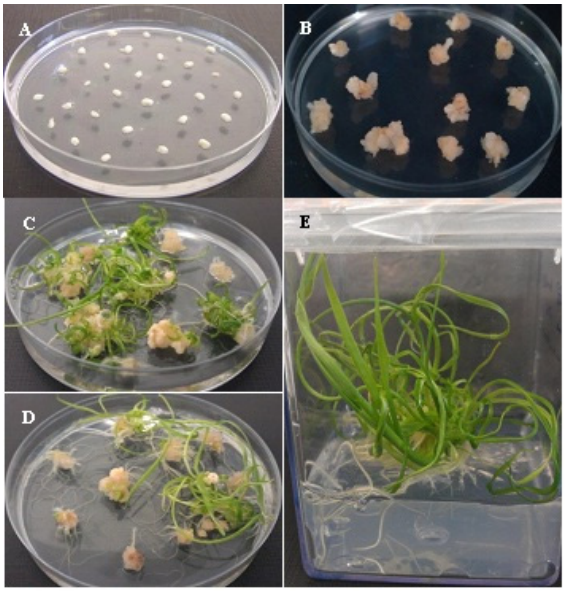

Fig. 1 Callus induction and plantlet regeneration from durum wheat MEs and ImEs. (A) Embryos in induction media, (B) callus formation after 4 weeks of culture, (C) plantlet regeneration from ImEs after transfer to regeneration media, (D) plantlet regeneration from MEs after transfer to regeneration media, and (E) rooting. ImE: immature embryo; ME: mature embryo

Table 1 ANOVA[a] for effects of variety, medium, and variety-medium interaction on callus weight, plantlet regeneration (%), NPR/Cal[b], and NPR/RCal[c] in durum wheat from MEs[d] and ImEs[e]

	Callus weight (g)		Plantlet regeneration (%)		NPR/Cal		NPR/RCal	
Source	ME	ImE	ME	ImE	ME	ImE	ME	ImE
Variety	8.18***	9.37***	18.22***	4.7**	8.72***	4.67**	3.64*	3.51*
Medium	68.61***	46.40***	34.97***	25.45***	13.86***	6.94**	6.26**	0.72
Variety × medium	2.31*	4.27**	3.65**	2.12	1.66	2.62*	0.95	6.13***

[a]ANOVA: analysis of variance.
[b]NPR/Cal: number of plantlets regenerating per callus.
[c]NPR/RCal: number of plantlets regenerated per regenerating callus.
[d]ME: mature embryo.
[e]ImE: immature embryo.
*Significant at $p < 0.05$; **significant at $p < 0.01$; ***significant at $p < 0.001$.

Table 2 Mean callus weight of MEs[a] and ImEs[b] from four durum wheat varieties obtained on three induction and maintenance culture media after 4 weeks of culturing and their effects on plantlet regeneration (%), NPR/Cal[c], and NPR/RCal[d]

	Callus weight (g)		PCI[e] (%)		Plantlet regeneration (%)		NPR/Cal		NPR/RCal	
	ME	ImE	ME	ImE	ME	ImE	ME	ImE	ME	ImE
Variety										
Amria	2.20^{f}	3.02^{f}	100^{g}	100^{g}	17.54^{g}	26.59^{f,g}	0.40^{f,g}	0.78^{g}	1.97^{f,g}	2.78^{g}
Chaoui	2.06^{f}	3.09^{f}	100^{g}	100^{g}	14.08^{g}	29.85^{g}	0.27^{f}	0.78^{g}	1.42^{f,g}	2.99^{g}
Marouane	2.78^{g}	3.73^{g}	100^{g}	100^{g}	15.29^{g}	36.44^{g}	0.54^{g}	1.06^{g}	2.78^{g}	2.57^{f}
Tomouh	2.20^{f}	2.50^{h}	100^{g}	100^{g}	1.84^{f}	18.33^{f}	0.04^{h}	0.37^{f}	0.60^{f}	1.77^{f}
CD[i]	0.32	0.46	0	0	4.69	10.12	0.20	0.37	1.37	0.81
Medium*										
M1	2.81^{g}	3.06^{f}	100^{g}	100^{g}	13.66^{f}	31.96^{f}	0.39^{g}	0.73^{f,g}	2.26^{g}	2.58^{g}
M2	1.37^{f}	2.12^{h}	100^{g}	100^{g}	19.80^{g}	40.86^{g}	0.50^{g}	1.05^{g}	2.33^{g}	2.70^{g}
M3	2.73^{g}	4.07^{g}	100^{g}	100^{g}	3.09^{h}	10.59^{h}	0.05^{f}	0.45^{f}	0.49^{f}	2.30^{g}
CD	0.27	0.40	0	0	4.06	8.76	0.18	0.32	1.18	0.70

[a]ME: mature embryo.
[b]ImE: immature embryo.
[c]NPR/Cal: number of plantlets regenerating per callus.
[d]NPR/RCal: number of plantlets regenerated per regenerating callus.
[e]PCI: percentage of callus induction.
[f,g,h]The values followed by the same letter are not significantly different at α = 0.05 according to Duncan's multiple-range test.
[i]CD:
*M4: no callus formation.

showed higher callus weight for callus after 4 weeks of culture from mature and immature embryos (Table 2), the plantlet regeneration rates were lower from those calluses. On the other hand, using picloram auxin, which induced the least amount of callus weight, showed the highest plantlets regeneration percentage, indicating that picloram induces more embryogenic callus than 2,4-D. The durum wheat varieties used had a significant effect on plantlet regeneration ($p < 0.001$). In mature embryos, the varieties 'Amria', 'Chaoui' and 'Marouane' produced higher plantlet regeneration (17.54%), (15.29%), and (14.08%), respectively, compared to 'Tomouh' (1.846%). However, from immature embryos, 'Marouane' (36.44%) produced the highest plantlet regeneration, followed by 'Chaoui' (29.85%), 'Amria' (26.54%) and 'Tomouh' (18.33%) (Table 2).

For the mature embryos, the plantlet regeneration also varied significantly depending on the interaction of varieties and induction media ($p < 0.01$) (Table 1), the favorable medium was M2 supplemented with 2 mg/l picloram for the varieties 'Amria' (30.71%), 'Marouane' (23.57%) and 'Chaoui' (22.39%) and M1 supplemented with 2 mg/l 2,4-D for 'Tomouh' (3%) (Fig. 2). Using immature embryos, the favorable auxin was picloram for all varieties: 'Marouane' (60.16%); 'Chaoui' (41.33%); 'Amria' (34.51%) and 'Tomouh' (27.45%) (Fig. 2).

Number of plantlets regenerated per Callus

The number of plantlets regenerated per callus was significantly affected by variety and the auxin used (Table

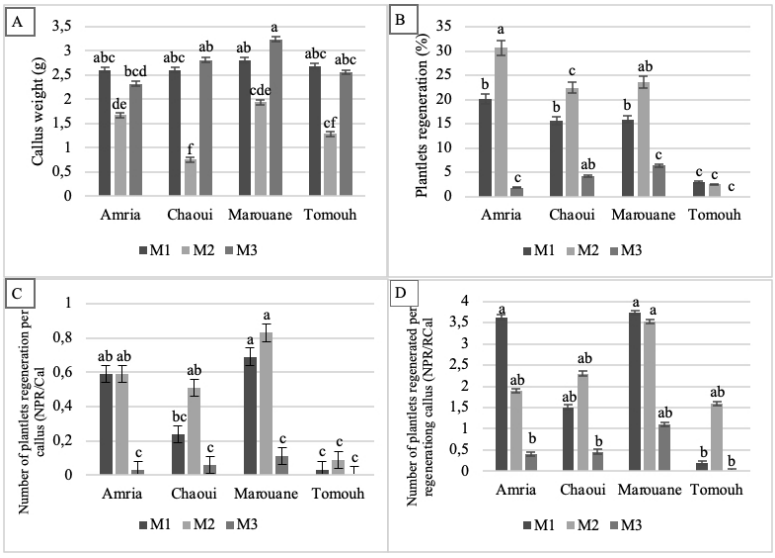

Fig. 2 Effect of culture medium and variety on (A) callus weight, (B) plantlet regeneration, (C) NPR/Cal, and (D) and NPR/RCal of MEs of durum wheat. Use of the same letter "a," "b," and/or "c" above the bar indicates that the results are not significantly different at α = 0.05 according to the LSD test. LSD: least significant difference according to the *t*-test; ME: mature embryo; NPR/Cal: number of plantlets regenerating per callus; NPR/RCal: number of plantlets regenerated per regenerating callus

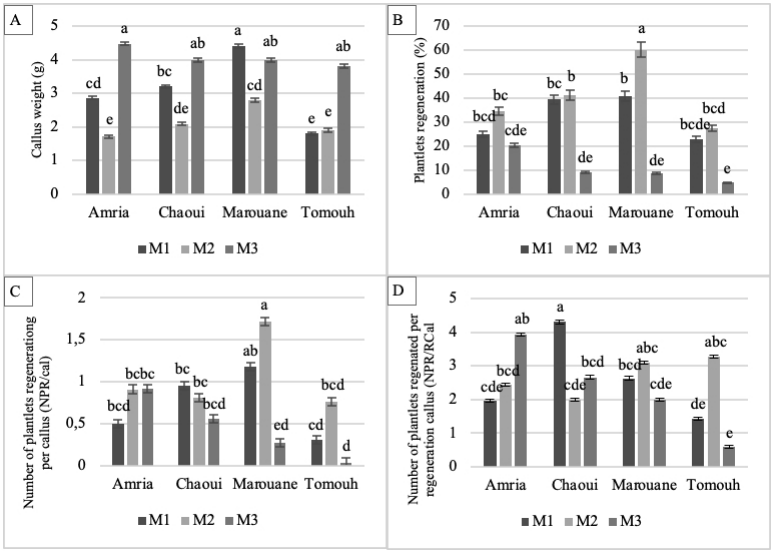

Fig. 3 Effect of culture medium and variety on (A) callus weight, (B) plantlet regeneration, NPR/Cal, and (D) NPR/RCal of ImEs of durum wheat. Use of the same letter "a," "b," "c," and/or "d" above the bar indicates that the results are not significantly different at α = 0.05 according to the LSD test. LSD: least significant difference according to the *t*-test; ME: mature embryo; NPR/Cal: number of plantlets regenerating per callus; NPR/RCal: number of plantlets regenerated per regenerating callus

1). Among mature embryos, 'Marouane' had the highest number of plantlets per callus (0.54), followed by 'Amria' (0.40), 'Chaoui' (0.27), and 'Tomouh' (0.04). From immature embryos, 'Marouane', 'Amria' and 'Chaoui' produced a higher number of plantlets per callus, 1.06, 0.78 and 0.78, respectively, across different induction media. 'Tomouh' (0.37) produced significantly fewer plantlets per callus (Table 2). Concerning the mature embryos, the use of picloram and 2,4-D as auxin gave a higher number of regenerated plantlets per callus (0.5); (0.39), respectively, and M3 media had the lowest value (0.05). From immature embryos, picloram and 2,4-D were favorable for the regeneration of more plantlets, with 1.05 and 0.73, respectively. The number of regenerated plantlets per callus was also influenced by variety, medium interaction (Table 1). The use of picloram gave the highest value for all varieties tested, 'Marouane' (1.72); 'Amria' (0.91), 'Chaoui' (0.81) and 'Tomouh' (0.76) for both mature (Fig. 2) and immature embryos (Fig. 3).

Table 3 ANOVA[a] for effects of variety, explant, and variety-explant interaction on callus weight, plantlet regeneration (%), NPR/Cal[b], and NPR/RCal[c] in durum wheat from MEs[d] and ImEs[e]

	Callus weight (mg)	Plantlet regeneration (%)	NPR/Cal	NPR/RCal
Source				
Embryo	60.85***	63.35***	33.06***	8.88**
Variety	15.37***	12.18***	10.89***	5.377**
Embryo × variety	2.55	1.61	0.38	1.84

[a]ANOVA: analysis of variance.
[b]NPR/Cal: number of plantlets regenerating per callus.
[c]NPR/RCal: number of plantlets regenerated per regenerating callus.
[d]ME: mature embryo.
[e]ImE: immature embryo.
**Significant at $p < 0.01$; ***significant at $p < 0.001$.

Table 4 Mean callus weight and plantlet regeneration, NPR/Cal[a], and NPR/RCal[b] of MEs[c] and ImEs[d] from four durum wheat varieties

	Callus weight (g)	Plantlet regeneration (%)	NPR/Cal	NPR/RCal
Embryo				
ImEs	3.08[e]	27.80[e]	0.74[e]	2.53[e]
MEs	2.30[f]	12.19[f]	0.31[f]	1.69[f]
LSD[g]	0.21	3.89	0.14	0.55

[a]NPR/Cal: number of plantlets regenerating per callus.
[b]NPR/RCal: number of plantlets regenerated per regenerating callus.
[c]ME: mature embryo.
[d]ImE: immature embryo.
[e,f]The values followed by the same letter are not significantly different at α = 0.05 according to Duncan's multiple-range test.
[g]LSD: least significant difference according to the *t*-test.

Number of plantlets regenerated per regenerating callus

The number of plantlets regenerated per regenerating callus is affected by the genotype and media (Table 1). Immature embryos treated with picloram and 2,4-D have the highest NPR/RCal (more than 2) (Table 2). On M1 media, the NPR/RCal was highest for variety 'Chaoui' (4.31); on M3 media, it was highest for 'Amria' (3.94); and on M2 media, it was highest for 'Marouane' (3.1) and 'Tomouh' (3.28) (Fig. 3).

Comparison of two different explant sources

The type of explant used had a significant effect on callus weight and plantlet regeneration ($p < 0.001$) (Table 3). Callus weight from immature embryos (3.08 g) was significantly higher than that from mature embryos (2.30 g) (Table 4). The highest regeneration percentage (27.8%) was obtained with immature embryos explants compared to mature embryos (12.19%).

Discussion

In this study, we explored the effects of auxin 2,4-D, picloram and their combination on callus formation and plantlet regeneration using mature and immature embryo explants of four Moroccan durum wheat varieties: 'Amria', 'Chaoui', 'Marouane', and 'Tomouh' (Table 1). We were able to determine which media are favorable for each variety, which will allow us to use mature and immature embryos as explants in regenerating and transforming durum wheat *in vitro*.

In the present study, the results showed that medium, variety, and medium-variety interaction strongly affected callus production (Table 2), which confirm other studies results showing that medium components and genotype greatly influence callus induction in bread wheat and durum wheat. (Mahmood and Razzaq 2017; Ren et al. 2010; Senhaji et al. 2021; Wang et al. 2018). The difference in embryogenic competence in plants or genotypes can be partly caused by the effect of gene action of the plant genotype and also may be related to the effect of the variation of endogenous auxin levels (Jiménez 2005). In

wheat, embryogenic calli normally have a higher concentration of endogenous auxins than non-embryogenic calli (Jiménez and Bangerth 2001). Auxin is one of the primary factors governing plant regeneration in plant tissue culture and especially in durum wheat. In most studies of culture from mature ~~embryos~~ and immature embryos of durum wheat and other cereals, 2,4-D is the most commonly used exogenous growth regulator for wheat (Ekom et al. 2013; Miroshnichenko et al. 2009).

In previous studies, 2,4-D was used for callus induction at concentrations ranging from 0.5 to 6 mg/l in hexaploid wheat (Miroshnichenko et al. 2016; Yadav et al. 2020) and 1, 2 and 5 mg/l in durum wheat (Borrelli et al. 1991). For scutellum induction and inflorescence explants of durum wheat, He and Lazzer (2001) used picloram at concentrations of 2, 4, and 6 mg/l and 2,4-D at concentrations of 1, 2, and 4 mg/l. Their results revealed that picloram significantly increases the plant regeneration frequency of both explant types more than 2,4-D. The same result was observed in bread wheat (Barro et al. 1998). Another study conducted by Miroshnichenko et al. (2016) used picloram and 2,4-D at a concentration ranging from 1 to 6 mg/l for immature embryos of tetraploid and hexaploid wheat, their results suggest that induction media must contain picloram to achieve the maximum amount of plant regeneration in hexaploid wheat. However, for tetraploid wheat T timopheevii, the plant regeneration frequency increased significantly using 2,4-D instead of picloram at a concentration of 2 mg/l, and a similar regeneration coefficient was obtained with 3 mg/l of 2,4-D, 4 mg/l of picloram, or 4 mg/l of dicamba.

In our experiments, picloram was found to be a better auxin that induces plant regeneration from durum wheat cultures than 2,4-D and the combination of both auxins. This result was in agreement with the results of the studies conducted on bread wheat (Barro et al. 1998), hexaploid T. *kiharae* (Miroshnichenko et al. 2016), and durum wheat (He and Lazzeri 2001). In contrast, Mendoza and Kaeppler (2002) demonstrated that picloram-containing media showed the highest callus growth from mature embryos of bread wheat but also gave the lowest mean number of plants regenerated compared to 2,4-D. Another study achieved by Barro et al. (1999) reported that regeneration of immature inflorescence induced by picloram was almost twice as great as the regeneration of immature inflorescence induced by 2,4-D. In contrast, the scutellum regeneration frequencies were higher using 2,4-D.

Furthermore, NPR/Cal varied significantly depending on varieties and media, for both mature and immature embryos. ‘Marouane’ recorded the highest rate followed by ‘Amria’, ‘Chaoui’ and ‘Tomouh’, whereas M2 media has the highest rate of NPR/Cal. However, another study reported dicamba-based treatments produced the highest mean number of plantlets regenerated per embryo cultured (2.5), followed by 2,4-D (0.8) and then picloram (0.5) (Mendoza and Kaeppler 2002).

We found that the plantlet regeneration from immature embryos was significantly higher than mature embryos. Our study correlated with Wang et al. (2014) and Ahmadpour et al. (2018) that reported that immature embryos are the explant of choice for wheat tissue culture trials due to their high regeneration competency. In contrast, Özgen et al. (1996) reported that mature embryos of durum wheat induced by 2,4-D had a low frequency of callus formation but a higher regeneration capacity as compared to immature embryos.

Conclusions

Based on the obtained results, we recommend the use of picloram as auxin instead of 2,4-D due to its positive effect in increasing the *in vitro* plant regeneration of Moroccan durum wheat varieties; which are the most recalcitrant. This auxin is highly recommended to ~~will~~ be used in genetic transformation of immature and mature embryos.

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