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*Original Research*

# Induced dedifferentiation of barley (*Hordeum vulgare* L.) embryonic cells and its relationship with agronomic traits

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**Abstract:** This study was carried out to investigate the response of 42 Iranian and European barley (*Hordeum vulgare* L.) cultivars to induced dedifferentiation of embryonic cells via immature embryo culture and understand the relationship between embryo culture characters and agronomic traits. The cultivars were evaluated for dedifferentiation of embryonic cells or callus induction from immature embryo culture based on a completely randomized design with unequal replication. Immature embryos were placed scutellum down on cell dedifferentiation medium based on MS and supplemented with 2.5 mg/l 2,4-D. The developed calli were transferred to MS regeneration medium with different concentrations and combinations of plant growth regulators. The results of group comparisons showed that Iranian cultivars were greater than European cultivars regarding callus growth rate, callus primary diameter and total regenerated plantlets. The path correlation analysis revealed that grain width and kernel filling period had the highest positive and negative direct effects on embryo culture traits, respectively. Clustering cultivars based on the embryo culture characters and agronomic traits divided the cultivars into three groups. The third group consisted of the cultivars which all of them were with the highest mean for flag leaf length, days to anthesis, grain yield, callus growth rate and callus primary diameter. Mantel test revealed a negative (-0.101) and significant correlation ($P<0.01$) between embryo culture characters and agronomic traits. The significant relationships between few numbers of embryo culture characters and agronomic traits confirm that these characteristics could be genetically dependent and also tissue culture characters can be estimated from agronomic data.

***Key words:*** Cell dedifferentiation; *Hordeum vulgare* L.; Embryo culture; Path analysis; Plantlet regeneration.

## Introduction

Barley (*Hordeum vulgare* L.) is one of the world's major cereal crops ranking fourth behind wheat, rice and maize in terms of agronomic importance (1). Barley improvement through genetic transformation and *in vitro* techniques requires the establishment of an efficient and reproducible plantlet regeneration system (1, 2). These techniques have the potential to assist in breeding improved barley cultivars. *In vitro* cultures offer the possibility of controlling and measuring precisely for growth and development of plant tissues (3). The regeneration of different plant cells from callus of *Hordeum vulgare* is a major step in obtaining new lines (4, 5). Plant production via tissue culture has advantages over traditional propagation methods because it leads to the production of disease and virus–free plants. Also, it allows the production of a high number of plants, in a short time period and in a limited propagation space. In addition, rapid multiplication rate of plants that is difficult to propagate conventionally can be easily achieved via *in vitro* culture (6). Immature embryos are the most responsive source to produce embryogenic callus and regenerated plantlets among other explants in culture (7). Also, callus formation is a desirable prerequisite for plantlet regeneration because callus offers the greatest opportunity for *in vitro* selection and production of genetic variations (4, 8). Moreover, callus can be used as a source of protoplasts suspension cultures and as a reliable tissue for particle bombardment and genetic transformations (9). Callus induction and plantlet regeneration potential are highly dependent upon the genotype and level of hormone in the culture media (8, 10). Regenerated plants are expected to have the same genotype as the mother plant. However, in some cases phenotypic and cytological variants have been found among regenerated barley plants (11). In barley, differences in the production of embryogenic calli and regenerated plants have been observed when cultures were initiated from immature scutella, leaf bases/apical meristems (12), mature embryos (13), immature embryos (2) and immature scutella (14). But immature zygotic embryos are currently the most reliable and efficient target tissue for *in vitro* regeneration of cereals, this has already been well documented in some reports by Dahleen and Bregitzer (2002) and Chang *et al.* (2003) (2, 15). The genotype of donor plants and growth characteristics of induced calli are the most important factors affecting plantlet regeneration (16). In cereals, whereas mature embryos are easily accessible without any restriction in time but callus is used less by lower generation. The amount of callus induction and plantlet regeneration in tissue culture of barely are commonly influenced by the explants source (15, 17, 18), genotype (19) and culture medium (20), among which genotype is often the dominant one. Unfortunately, those genotypes which have had suitable tissue culture responses cannot be determined before testing in experiments, because there is

very little information about related mechanisms. The results of the most studies showed that tissue culture traits are quantitative trait loci (QTLs) (21). Haliloglu *et al.,* (2005) attempted to predict the outcome of tissue culture in advance by correlating some agronomic traits to crucial tissue culture traits (22). Dodig *et al.* (2008) and Li *et al.* (2003) have tried to find the relationship between tissue culture and agronomic traits of wheat in the detail (23, 24). They concluded significant relationship of callus formation to kernel numbers per spike and effective spikelet as well as callus weight to tillers per plant and kernel number per spikelet. So, relationships may be due to the fact that tissue culture traits might be controlled by the genetic system controlling the incidence of agronomic traits or partial linkage with them. The aims of the current study were to evaluate the response of Iranian and European barley cultivars to induced dedifferentiation of embryonic cells via immature embryo culture, to determine the embryo culture characters and agronomic traits, which can be related to each other in barley genotypes and to develop the supposition which tissue culture characters can be estimated from agronomic data.

## Materials and Methods

### Plant materials

In this investigation, 42 Iranian and European barley (*Hordeum vulgare* L.) cultivars received from Natural Resources & Agricultural Research Center in Kermanshah, Iran and Genomics Research Centre (CRA-GPG) in Italy were used (Table 1).

### Plant growth and agronomic traits measurements

The barley cultivars were grown on the basis of a randomized complete block design with three replications during 2013-2014 in the research field of Razi University, Kermanshah in the west of Iran (latitude 34° 19′ N, longitude 47° 7′E, and altitude 1322 m). Each plot consisted of five rows whit a sowing density of 350 seeds per $m^2$. Rows were 3 cm long and separated by 22.5 cm from each other. The following characters were determined: flag leaf length (FLL), number of kernel per spike (NKPS), plant height (Phe), awn length (AL), grain length (GL), grain width (GW), days to heading (DTH), days to anthesis (DTA), grain yield (GY), kernel filling period (KFP) and thousand kernel weight (TKW).

### Dedifferentiation of embryonic cells via immature embryo culture and plantlet regeneration

The spikes were collected from field-grown plants approximately 14-16 days after anthesis. The immature caryopses were sterilized in 70% ethanol for 1 min and were soaked in 2% sodium hypochlorite (commercial Clorox) supplemented with few drops of Tween 20 for 10 min, Ebtissam *et al.* (2003) (25). Then they were rinsed by double distilled water for three times. Immature zygotic embryos were dissected out aseptically and used as explants. Ten explants were placed scutellum down on Murashige and Skoog (1962) MS medium containing 2.5 mg/l 2,4-D, in a 90 × 10-mm Petri dish (26). The cultures were incubated in darkness at 26°C for four weeks. The callus induction percentage (CIP), callus growth rate (CGR), callus primary diameter (CPD), callus fresh weight (CFW) and relative water content (RWC) of callus were measured. Three different regeneration media (MS+1 mg/l 2,4-D + 0.5 mg/l BAP; MS+ 0.1 mg/l BAP; MS+ 1 mg/l 2,4-D + 2 mg/l BAP) were used for evaluation of the regeneration characters including the percentage of rooted, shooted, complete plantlet and total regenerated plantlets. All cultures were maintained at a temperature of 26°C under 16-h photoperiod for four weeks.

### Statistical analysis

The experiment of cell dedifferentiation or callus induction was carried out using a completely randomized design with three to five replications. Also, the regeneration experiment was factorial arranged in a completely randomized design with two factors and three replications. The factors were barley cultivar (42 cultivars) and regeneration medium (three different media). Relationships between every possible pair of various agronomic and tissue culture traits were determined by Pearson correlation analysis. The path coefficient analysis was performed to examine the relationship between tissue culture and agronomic traits. The Ward's method was used to form hierarchical clustering (27). In order to study the relationships among of the embryo culture characters and agronomic traits with respect to "distances matrix" was used of Mantel test (28). The statistical analyses were performed with the SAS (Ver. 9.1), SPSS (Ver. 16.0.1, SPSS Inc) and XLSTAT (Ver. 2015. 01) software and the means were compared using the Duncan test ($p < 0.05$).

## Results

### Analysis of variance for embryo culture characters in cell dedifferentiation stage

Analysis of variance showed highly significant differences ($P<0.01$) among the cultivars for callus growth rate (CGR) and callus primary diameter (CPD) (Table 2). Of course, the callus induction percentage from the immature embryo was 100% for all cultivars (Fig. 1).

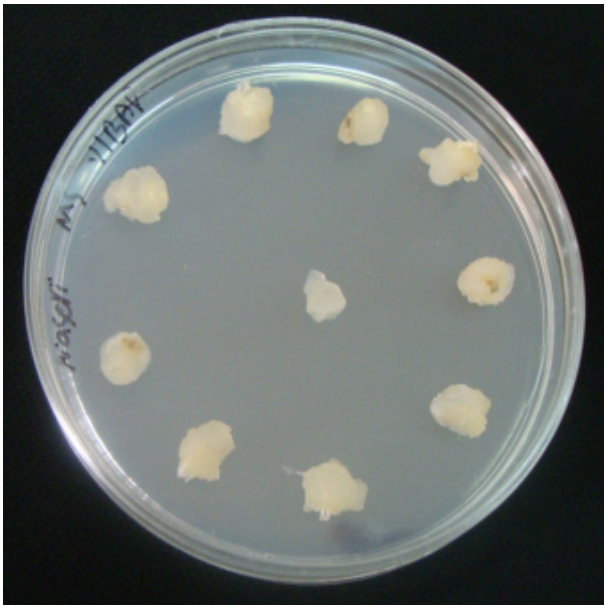

**Figure 1.** Cell dedifferentiation in barley immature embryos.

**Table 1.** The names and characteristics of the used barley cultivars.

| Cultivars No. | cultivars name | Geographic Location | Pedigree | Growth habit | Row type | Grain type | Time of sowing |
|---|---|---|---|---|---|---|---|
| 1 | Karoon | Iran | Strain- 205 | F | 6 | Hulled | Winter |
| 2 | Afzal | Iran | Chahafzal | F | 6 | Hulled | Winter |
| 3 | Sahra | Iran | L. B. LRAN/ Una8271// Giorias"s" Com | F | 6 | Hulled | Winter |
| 4 | Nosrat | Iran | Karoon/Kavir | F | 6 | Hulled | Winter |
| 5 | Makooei | Iran | Star | F | 6 | Hulled | Winter |
| 6 | Mahor | Iran | Wi2291/Wi2269//Er/Amp | W | 2 | Hulled | Winter |
| 7 | Fajr30 | Iran | Lignee131/ Gerbet//Alger- Ceres/ jonoob | F | 6 | Hulled | Winter |
| 8 | Yoosef | Iran | Lignee527/chn-01//Gustoe/4/Rhn-08/3/ DeirAlla 106//DI71/strain 205 | S | 6 | Hulled | Winter |
| 9 | Zarjo | Iran | 1-28-9963 | F | 6 | Hulled | Winter |
| 10 | Aras | Iran | Arumir | F | 2 | Hulled | Winter |
| 11 | Nimrooz | Iran | Trompillo, CMB74A-432-25B-1Y-IB-IY-OB | F | 2 | Hulled | Winter |
| 12 | Gorgan4 | Iran | Herta | F | 2 | Hulled | Winter |
| 13 | Sararood | Iran | Chicm/An57//Albert | W | 2 | Hulled | Winter |
| 14 | Jo Danmar | Iran | Denmark55 | W | 2 | Hulled | Winter |
| 15 | Jonoob | Iran | Gloria" s"/ Copal"s" | F | 6 | Hulled | Winter |
| 16 | Reyhan | Iran | Rihane-03/4Alanda/lLignee5 27/Arar/3/ Centinela/2* | S | 6 | Hulled | Winter |
| 17 | Airone | Europe | Gitane x FIOR 763 | W | 2 | Naked | Winter |
| 18 | Aqvirone | Europe | FIOR 5186 x Naturel | W | 2 | Hulled | Winter |
| 19 | Sirio | Europe | FIOR 2136 x Arco | W | 2 | Hulled | Winter |
| 20 | Astartis | Europe | (IABO x Arda3) x Amillis | W | 2 | Naked | Spring |
| 21 | Zacinto | Europe | IABO 329 x Arda | W | 2 | Naked | Spring |
| 22 | Scirocco | Europe | FIOR 1000 x Express | S | 6 | Hulled | Winter |
| 23 | Tidone | Europe | (Okos x 273 cat.) x Igri | S | 2 | Hulled | Winter |
| 24 | Doria | Europe | (Nure x Zita)x(Nure x PO 202.169) | W | 2 | Hulled | Winter |
| 25 | Martino | Europe | FIOR 3007 x Federal | W | 6 | Hulled | Winter |
| 26 | Sfera | Europe | ((Katy x HJ54/30) x Igri x Arda) x (Tipper x Sonja)) x Amillis | W | 2 | Hulled | Winter |
| 27 | Paragelia | Europe | Airone x Arco | W | 2 | Hulled | Winter |
| 28 | Arda | Europe | Igri x HJ 51-15-3 | W | 2 | Hulled | Winter |
| 29 | Alce | Europe | (Tipper x Igri3) x [(Tipper x Alpha)x(Sonja x Wb117/18)] | W | 2 | Hulled | Winter |
| 30 | Trebbia | Europe | selection from FiorSynt 3 | W | 6 | Hulled | Winter |
| 31 | Cometa | Europe | PO202.169 x FO 3358 | W | 2 | Hulled | Winter |
| 32 | Ponente | Europe | (Vetulio x Arma) x Express | W | 6 | Hulled | Winter |
| 33 | Rodoroz | Europe | Baraka x Gotic | W | 2 | Hulled | Winter |
| 34 | Alfeo | Europe | Tipper x Igri | W | 2 | Hulled | Winter |
| 35 | Aliseo | Europe | (Plaisant x Gerbel) x Express | W | 6 | Hulled | Winter |
| 36 | Vega | Europe | Rebelle x FIOR 1341 | W | 6 | Hulled | Winter |
| 37 | Panaka | Europe | Amillis x Diadem | W | 2 | Hulled | Winter |
| 38 | Alimini | Europe | FIOR 2551 x Federal | W | 6 | Hulled | Winter |
| 39 | Explora | Europe | [(Onice\Arma\\Onice\Mirco\\\Jaidor) x (Plaisant\Jaidor\Express)] x Gotic | W | 6 | Hulled | Winter |
| 40 | Aiace | Europe | FO 1078 x FO 1638 | W | 2 | Hulled | Winter |
| 41 | Nure | Europe | (FIOR 40 x Alpha2) x Baraka | W | 2 | Hulled | Winter |
| 42 | Aldebaran | Europe | Rebelle x Jaidor | W | 6 | Hulled | Winter |

**F**: Facultative; **W**: Winter; **S**: Spring.

**Table 2.** Analysis of variance for the measured traits in callus induction stage from immature embryo of 42 barley cultivars.

| | | Mean of squares | | | |
|---|---|---|---|---|---|
| **Source of variation** | **df** | **Callus growth rate (CGR) mm** | **Callus primary diameter (CPD) mm** | **Callus fresh weight (CFW) gr** | **Relative water Content of callus (RWC) %** |
| Cultivar | 41 | 11.917 ** | 21.330 ** | 0.179 ns | 0.971 ns |
| Residual | 160 | 0.443 | 2.283 | 0.134 | 0.729 |
| CV(%) | | 7.75 | 12.43 | 3.02 | 18.82 |

** Significant at 1% level of probability; ns: not significant.

**Table 3.** Analysis of variance for the measured traits in regeneration stage.

| Source of variation | df | Mean of squares | | | |
|---|---|---|---|---|---|
| | | Shooted plantlet (%) | Rooted plantlet (%) | Complete plantlet (%) | Total regenerated plantlets (%) |
| Cultivar | 41 | 0.006 $^{ns}$ | 0.005 $^{ns}$ | 0.046 $^{ns}$ | 0.162 $^{**}$ |
| Plant growth regulators | 2 | 0.547 $^{**}$ | 0.242 $^{**}$ | 0.803 $^{**}$ | 4.126 $^{**}$ |
| Plant growth regulators×Cultivar | 82 | 0.0123 $^{ns}$ | 0.011 $^{ns}$ | 0.038 $^{ns}$ | 0.073 $^{ns}$ |
| Residual | 252 | 0.106 | 0.009 | 0.050 | 0.055 |
| CV(%) | | 18.9 | 18.1 | 56.8 | 56.3 |

** Significant at 1% level of probability; ns: not significant.

**Figure 2**. Plantlet production in regeneration stage in barley.

## Analysis of variance for embryo culture characters in plantlet regeneration stage

The regeneration of dedifferentiated cells or callus derived from barley immature embryo was shown in Figure 2. The calli derived from immature embryos of barley were regenerated to rooted, shooted and complete plantlet. The all evaluated traits in regeneration stage were influenced by the plant growth regulators (Table 3). A significant difference was only observed among cultivars for the percentage of the total regenerated plantlets.

## Mean comparison of barley cultivars in cell dedifferentiation and regeneration stages

The results of the mean comparison of the barley cultivars showed that callus growth rate (CGR) and callus primary diameter (CPD) were the highest in cultivars Afzal, Karoon, Martino and Astartis and were the lowest in cultivars Explora, Aldebaran and Zarjo (Table 4). The cultivars Astartis and Afzal had the highest percentage of total regenerated plantlets (TRP) from immature embryos (80% and 64%, respectively) and cultivar Makooei had the lowest percentage of total regenerated plantlets (TRP). Mean comparison of plant growth regulators in the regeneration stage showed that level of 0.1 mg/l BAP was the highest for percentage of complete plantlet and total regenerated plantlets, while 1 mg/l 2,4-D + 0.5 mg/l BAP and 1 mg/l 2,4-D + 2 mg/l BAP were the highest for shooted plantlet and rooted plantlet, respectively (Fig. 3).

The results of group comparisons in cell dedifferentiation (callus induction) and regeneration stages showed that Iranian (vs European) cultivars, Iranian 6-rowed (vs European 6-rowed) cultivars, Iranian 2-rowed (vs Iranian 6-rowed) cultivars, autumn (vs spring) cultivars and 2-rowed (vs 6-rowed) cultivars had superior callus growth rate, callus primary diameter and total regenerated plantlets (Table 5).

## Relationship analysis between embryo culture characters and agronomic traits

The Pearson correlation coefficient (r) was considered between embryo culture and agronomic traits of 42 barley cultivars in Table 6. The results of this study showed that callus growth rate (CGR) was positively related to callus primary diameter (CPD) and callus fresh weight (CFW). The percentage of complete plantlets and percentage of total regenerated plantlets had negatively significant correlations with the percentage of rooted plantlets and positively significant correlations with shooted plantlets. This study showed that callus fresh weight (CFW) and relative water content of callus (RWC) were negatively related to the kernel filling period (KFP). A positive significant correlation was observed between callus primary diameter (CPD) and grain width (GW).

Direct and indirect effects for path coefficient analysis of agronomic traits on embryo culture characters are shown in Table 7. The path analysis revealed that grain width had the highest positive direct effect on callus primary diameter (0.71). The kernel filling period had the highest negative direct effect on callus fresh weight (-0.67).

## Cluster analysis and Mantel test

Cluster analysis based on the embryo culture and agronomic traits divided the cultivars into three groups (Fig. 4). The first group was including a large number

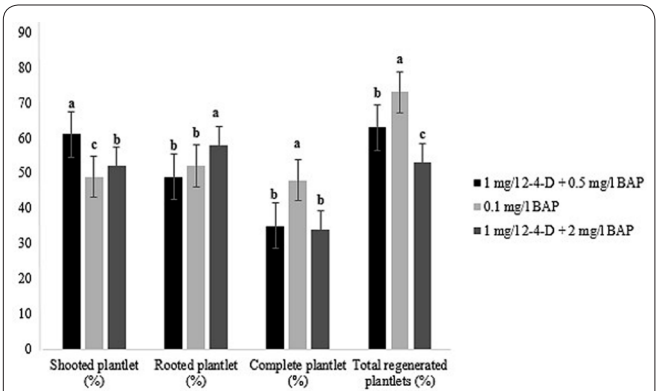


**Figure 3.** Mean comparison of the effect of plant growth regulators on regeneration characteristics in barley.

**Table 4.** Mean comparison of the barley cultivars for the measured traits in immature embryo culture.

| Name of cultivar | Callus growth rate (CGR) (mm) | Callus primary diameter (CPD) (mm) | Total regenerated plantlets (TRP) % |
|---|---|---|---|
| Karoon | 10.58 ab | 15.46 ab | 53 a-d |
| Afzal | 10.78 a | 16.02 a | 64 a-d |
| Sahra | 9.88 a-f | 14.04 a-e | 33 a-e |
| Nosrat | 10.10 a-e | 14.09 a-e | 48 a-d |
| Makooei | 7.97 i-l | 11.35 f-l | 13 a |
| Mahor | 9.77 b-f | 13.73 a-f | 48 a-d |
| Fajr30 | 10.13 a-e | 14.16 a-e | 37 a-e |
| Yoosef | 9.49 c-g | 13.52 b-g | 27 cde |
| Zarjo | 9.31 efg | 12.80 b-i | 33 a-e |
| Aras | 8.52 g-j | 11.83 e-k | 44 b-e |
| Nimrooz | 9.18 e-h | 12.45 d-j | 60 abc |
| Gorgan4 | 9.74 b-f | 13.92 a-e | 51 b-e |
| Sararood | 10.36 a-d | 14.24 a-d | 62 abc |
| JoDanmark | 8.90 f-i | 11.95 d-k | 26 cde |
| Jonoob | 9.83 a-f | 13.98 a-e | 22 de |
| Reyhan | 9.02 f-h | 12.62 d-i | 51 a-d |
| Airone | 9.57 c-f | 13.66 b-f | 31 a-e |
| Aqvirone | 6.44 mn | 9.97 klm | 37 b-e |
| Sirio | 7.62 jkl | 10.58 i-m | 26 cde |
| Astartis | 10.43 abc | 15.01abc | 80 a |
| Zacinto | 8.96 fgh | 12.06 d-k | 33 b-e |
| Scirocco | 7.68 j-l | 10.92 h-l | 28 b-e |
| Tidone | 9.24 e-h | 11.51 f-k | 57 a-d |
| Doria | 7.71 jkl | 11.22 g-l | 37 b-e |
| Martino | 10.56 abc | 15.46 ab | 53 a-d |
| Sfera | 6.64 mn | 9.69 k-n | 26 cde |
| Paragelia | 8.25 h-k | 11.51 f-k | 44 b-e |
| Arda | 9.00 fgh | 12.06 d-k | 37 b-e |
| Alce | 6.22 no | 9.05 l-n | 51 a-d |
| Trebbia | 7.98 i-l | 11.51 f-k | 42 b-e |
| Cometa | 7.14 lmn | 10.43 i-m | 48 a-e |
| Ponente | 5.42 o | 8.57 mno | 42 b-e |
| Rodoroz | 8.30 h-k | 11.52 f-k | 35 b-e |
| Alfeo | 9.74 b-f | 10.48 i-m | 33 b-e |
| Aliseo | 9.37 d-g | 13.28 b-h | 31 b-e |
| Vega | 6.376n | 9.69 k-n | 53 a-d |
| Panaka | 9.15 e-h | 12.45 d-j | 31 a-e |
| Alimini | 7.92 i-l | 11.22 g-l | 26 cde |
| Explora | 3.69 p | 6.79 o | 53 a-d |
| Aiace | 9.40 d-g | 13.46 b-g | 4 a-e |
| Nure | 9.56 c-f | 13.53 b-g | 35 b-e |
| Aldebaran | 4.64 mn | 7.64 no | 57 a-d |

Means that have at least one common letter didn't show significant difference in 5% level.

**Table 5.** The results of group comparisons of barley cultivars for the traits measured in callus induction and regeneration phases.

| | Comparison 1 | Comparison 2 | Comparison 3 | Comparison 4 | Comparison 5 |
|---|---|---|---|---|---|
| | Cultivars | 6-rowed cultivars | Iranian cultivars | Cultivars | Cultivars |
| | European vs Iranian | European vs Iranian | 6Rowed vs 2Rowed | Spring vs Autumn | 6Rowed vs 2Rowed |
| Callus growth rate (mm) | 8.32 ** 8.97 | 8.08 ** 8.62 | 8.6 ** 8.7 | 7.06 ** 8.68 | 0.39 ns 0.40 |
| Callus primary diameter (mm) | 11.7 ** 12.8 | 11.40 ** 12.24 | 12.2 ns 12.4 | 10.27 ** 12.20 | 0.3 ns 0.3 |
| Total regenerated plantlets (%) | 0.40 ns 0.38 | 0.23 ns 0.23 | 0.33 ns 0.32 | 0.1 ns 0.1 | 0.20 ** 0.34 |

ns and **: not significant and significant at p<0.01 respectively, for comparison 1 to5, separately.

**Table 6.** Pearson correlation coefficients among the investigated agronomic and embryo culture traits of 42 barley cultivars.

| | CGR | CPD | CFW | RWC | SsP | RP | CP | TRP | FLL | NKPS | Phe | AL | GL | GW | DTH | DTA | KFP | GY | TKW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CGR** | 1 | | | | | | | | | | | | | | | | | | |
| **CPD** | 0.947** | 1 | | | | | | | | | | | | | | | | | |
| **CFW** | 0.499** | 0.474** | 1 | | | | | | | | | | | | | | | | |
| **RWC** | 0.238 | 0.263 | 0.712** | 1 | | | | | | | | | | | | | | | |
| **ShP** | 0.266 | 0.243 | 0.233 | 0.017 | 1 | | | | | | | | | | | | | | |
| **RP** | 0.235 | 0.19 | 0.045 | 0.028 | 0.057 | 1 | | | | | | | | | | | | | |
| **CP** | -0.115 | -0.118 | -0.111 | -0.127 | 0.377** | -0.203* | 1 | | | | | | | | | | | | |
| **TRP** | -0.018 | 0.037 | -0.067 | -0.055 | 0.273** | -0.227* | -0.098 | 1 | | | | | | | | | | | |
| **FLL** | 0.078 | 0.081 | -0.009 | 0.022 | 0.085 | -0.076 | -0.004 | -0.106 | 1 | | | | | | | | | | |
| **NKPS** | 0.072 | 0.055 | -0.115 | -0.219 | 0.178 | -0.005 | -0.034 | -0.113 | 0.337* | 1 | | | | | | | | | |
| **Phe** | -0.064 | -0.001 | -0.001 | -0.056 | -0.026 | -0.079 | 0.052 | 0.093 | 0.535** | 0.369* | 1 | | | | | | | | |
| **AL** | -0.341* | -0.294 | 0.041 | -0.036 | -0.017 | -0.008 | -0.006 | 0.081 | -0.118 | -0.610** | 0.032 | 1 | | | | | | | |
| **GL** | -0.09 | -0.129 | 0.062 | -0.097 | 0.052 | -0.02 | -0.159 | -0.082 | 0.339* | 0.377* | 0.357* | -0.086 | 1 | | | | | | |
| **GW** | 0.214 | 0.309* | 0.199 | 0.323* | 0.087 | 0.091 | -0.113 | -0.178 | 0.096 | -0.630** | -0.024 | 0.385* | -0.039 | 1 | | | | | |
| **DTH** | -0.035 | 0.009 | -0.059 | -0.072 | 0.11 | -0.251 | -0.053 | -0.020 | 0.538** | 0.335* | 0.782** | 0.095 | 0.205 | 0.007 | 1 | | | | |
| **DTA** | -0.019 | 0.023 | -0.049 | -0.056 | 0.127 | -0.266 | -0.063 | -0.030 | 0.539** | 0.313* | 0.764** | 0.107 | 0.185 | 0.031 | 0.998** | 1 | | | |
| **KFP** | -0.337* | -0.3 | -0.437** | -0.417** | -0.112 | 0.133 | 0.065 | 0.101 | 0.116 | 0.318* | 0.364* | 0.123 | 0.396** | -0.134 | 0.359* | 0.334* | 1 | | |
| **GY** | -0.09 | -0.08 | -0.035 | -0.177 | 0.029 | -0.156 | -0.229 | -0.224 | 0.292 | 0.021 | 0.329* | 0.187 | 0.104 | 0.206 | 0.518** | 0.524** | 0.177 | 1 | |
| **TKW** | 0.109 | 0.142 | 0.079 | 0.135 | 0.054 | -0.145 | -0.085 | -0.120 | 0.104 | -0.501** | -0.122 | 0.215 | 0.091 | 0.755** | -0.012 | 0.006 | 0.014 | 0.365* | 1 |

*, ** Significantly at 5% and 1% level of probability, respectively. CGR: callus growth rate; CPD: callus primary diameter; CFW: callus fresh weight; RWC: relative water content of callus; ShP: Shooted plantlet; RP: Rooted plantlet; CP: Complete plantlet; TRP: total regenerated plantlets; FLL: flag leaf length; NKPS: number of kernel per spike; Phe: plant height, AL: awn length; GL: grain length; GW: grain width; DTH: days to heading; DTA: days to anthesis; GY: grain yield; KFP: kernel filling period; TKW: thousand kernel weight.

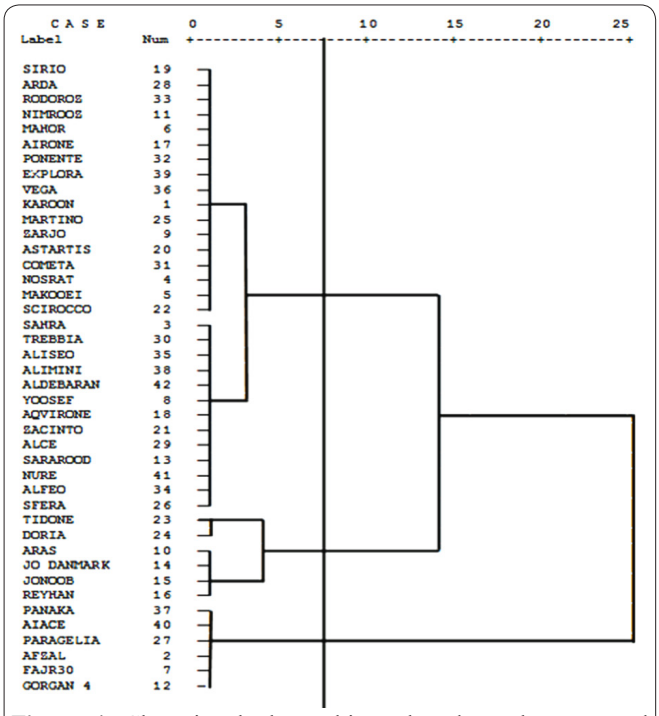


**Figure 4.** Clustering barley cultivars based on the measured embryo culture and agronomic traits via Ward method.

of cultivars with different characters. The second group consisted of the cultivars which all of them were with the lowest mean for flag leaf length, days to anthesis, grain yield, callus growth rate and callus primary diameter. The third group was opposite of the second group in their evaluated traits. Also, the calculated correlation by Mantel test between embryo culture characters and agronomic traits was negatively (-0.101) and significant ($P<0.01$) correlation.

## Discussion

Immature embryos are the dependable source of callus induction and regeneration potential efficiency in barley. Embryos culture of barley showed that genotype is an important success factor. The results of variance analysis confirmed previous findings reporting that response to immature embryo culture in barley (29, 30) and in durum wheat (7) is a genetically controlled phenomenon. Also, some researchers have suggested that genotype effect may be related to variations in endogenous hormone levels (31). Moreover, the effect of genotype and environment could influence the response of explants through the level of endogenous hormone (32). Serhantová *et al.* (2004) and Chang *et al.* (2003) reported callus induction percentage (100%) similar to the current study result (Fig. 1) (2, 4). Among different plant growth regulators, 0.1 mg/l BAP produced a high frequency of plantlet regeneration (Fig. 3). Probably, this is the reason that the low concentrations of BAP (0.5 mg/l and 1.0 mg/l) used on the embryos helped to create and develop a lot of lateral shoots (33). The most studies showed that effect of the plant growth regulators on callus induction is highly significant (34). The results of group comparisons showed that Iranian cultivars were greater than European cultivars regarding callus growth rate, callus primary diameter and total regenerated plantlets. The existence of significant differences among Iranian cultivars and European cultivars revealed that these cultivars have the considerable potential to utilize in breeding programs. This result demonstrates that there was noticeable genetic diversity, therefore it could be used as a suitable source for breeding programs.

The results showed that the cultivars with the great the callus diameter presented the high callus growth rate and relative growth rate (Tables 4 and 7). Ghasempour *et al.* (2008) reported a positive and significant correlation between callus growth rate (CGR) and the callus primary diameter (CPD) (35). In the most studies were observed the significant correlations between callus induction and plantlet regeneration traits (36, 37). Whereas, in the current study were not observed significant correlations between callus induction and plantlet regeneration traits (Table 7). The reason for this lack of relation could be related to those genes which control callus induction and plantlet regeneration. It is known that callus induction and plantlet regeneration capacity may be controlled independently of each other (31, 38).

Path analysis is a suitable method to find out direct and indirect effects on dependent traits (39). The linear

**Table 7.** Path coefficients (direct and indirect effects) of the agronomic traits on the embryo culture characters in 42 barley cultivars.

| Traits | Callus primary diameter (CPD) mm | | Callus fresh weight (CFW) gr | | Total regenerated plantlet (TRP) % | |
|---|---|---|---|---|---|---|
| | Direct | Indirect | Direct | Indirect | Direct | Indirect |
| FLL | 0.22 | -0.14 | -0.20 | 0.19 | 0.01 | -0.11 |
| NKPS | 0.53 | -0.47 | 0.32 | -0.44 | -0.57 | 0.46 |
| Phe | 0.07 | -0.07 | 0.15 | -0.15 | 0.39 | -0.30 |
| AL | -0.24 | -0.05 | 0.22 | -0.18 | -0.07 | 0.15 |
| GL | -0.17 | 0.04 | 0.24 | -0.17 | -0.09 | 0.01 |
| GW | 0.71 * | -0.40 | 0.21 | -0.01 | -0.58 | 0.40 |
| DTH | -1.23 | 1.24 | 0.37 | -0.43 | 0.20 | -0.22 |
| DTA | 1.32 | -1.29 | -0.34 | 0.29 | -0.20 | 0.17 |
| KFP | -0.25 | -0.05 | -0.67 ** | 0.23 | 0.15 | -0.05 |
| GY | -0.16 | 0.08 | -0.07 | 0.04 | -0.30 | 0.08 |
| TKW | 0.00 | 0.14 | 0.09 | -0.01 | 0.21 | -0.33 |

*,** Significantly at 5% and 1% level of probability, respectively. FLL: flag leaf length; NKPS: number of kernel per spike; Phe: plant height, AL: awn length; GL: grain length; GW: grain width; DTH: days to heading; DTA: days to anthesis; KFP: kernel filling period; GY: grain yield; TKW: thousand kernel weight.

relationship between variables is expressed by the correlation coefficient, which is stating the common variation coefficient of different traits (40). The significant correlations between the embryo culture characters and the agronomic traits were previously observed by Dodig *et al.* (2008), Li *et al.* (2003) and Haliloglu *et al.* (2005) (22, 23, 24). This will enable us to identify the superior genotypes for tissue culture response directly at the agronomic trait level. In confirmation of these results, Mantel test revealed similar assessments of genetic relationships among the barley cultivars by tissue culture characters and agronomic data. Barely has been potential plant for tissue culture (41-43).

In conclusion, the results of group comparisons showed that Iranian cultivars were greater than European cultivars regarding callus growth rate, callus primary diameter and total regenerated plantlets. This result demonstrates that there was noticeable genetic diversity; therefore it could be used as a suitable source for breeding programs. Lack of relation between cell dedifferentiation characters and plantlet regeneration traits is known that callus induction and plantlet regeneration capacity may be controlled independently of each other. The significant correlations between the tissue culture characters and the agronomic traits will enable us to identify the superior genotypes for tissue culture response directly at the agronomic trait level.

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**Interest conflict**
All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication.

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