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Dinamics of callusogenesis in Lancaster inbred lines

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Corn inbred lines in biotechnological and breeding investigations have to be sensitive to cultivation in vitro, to be able to proferently morphogenic calli and maintain their morphogenic potential during long period of time, to be able to regenerate and quickly accumulate fresh weight.

The dynamics of callusogenesis of ten inbred lines of *Zea mays L*. which belonged to commercial germplasm Lancaster was investigated. Among them DK267, DK212, DK6080, DK420-1 are originated from subgermplasm Oh43, DK633/266, DK298 – from subgermplasms Mo17, Oh43, DK633 – from subgermplasm Mo17, DK3070 – from subgermplasms Mo17, O92, DK236 – from subgermplasms Mo17, F2, DK633/325 – from subgermplasms Mo17, Mindszenpuszta. Inbred Chi31 (exotic germplasm) was taken as a standard.

Immature zygotic embryos, 1-1.5 mm in length, were harvested 10-12 days after self-pollination from field-grown plants and cultured on callus induction medium. This medium included N₆ inorganic components, 100 mg/l inositil, 100 mg/l casein hydrolysate, 10 mg/l AgNO₃, 690 mg/l L-proline, 1 mg/l 2,4-dichlorophenoxyacetic asid, 0,1 mg/l abscisic asid, 30 g/l sucrose. Embryos and calli were incubated under the temperature 27-25°C in the dark.

The frequencies of morphogenic callus initiation, initiation of callus type I and type II, frequency of spontaneous regeneration, frequency of brown explants and fresh weight callus at 30th and 60th days in culture (DIC) are present in tables 1, 2. These characteristics were calculated in ratio to total amount of responsive embryos (embryos with calli or swollen scutellia).

Table 1. Dynamics of callusogenesis of inbred lines in germplasm Lancaster

| Genotype | DIC | Number of cultivated embryos, among which | | Frequency of morphogenic callus initiation | Frequency of callus type I % | Frequency of callus type II, % |
|----------|-----|---|----------------------|--|------------------------------|--------------------------------|
| | | calluso- genetic | swollen scutellum | % | | |
| DK267 | 30 | 396 | 26 | $76,54 \pm 4,13$ | $72,75 \pm 4,34$ | $3,79 \pm 1,86$ |
| | 60 | 396 | 26 | $53,08 \pm 4,86$ | $48,34 \pm 4,87$ | $4,74 \pm 2,07$ |
| DK 212 | 30 | 224 | 0 | $44,67 \pm 6,38$ | $15,16 \pm 4,60$ | $29,51 \pm 5,85$ |
| | 60 | 224 | 0 | $13,39 \pm 4,56$ | 0 | $13,39 \pm 4,56$ |
| DK6080 | 30 | 184 | 0 | $38,04 \pm 7,18$ | 0 | $38,04 \pm 7,18$ |

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| | 60 | 184 | 0 | $3,26 \pm 2,63$ | 0.54 ± 1.09 | $2,72 \pm 2,40$ |
|-----------|----|-----|-----|------------------|------------------|------------------|
| DK420-1 | 30 | 184 | 0 | $32,61 \pm 6,93$ | $2,72 \pm 2,40$ | $29,89 \pm 6,77$ |
| | 60 | 184 | 0 | $2,17 \pm 2,16$ | $1,09 \pm 1,53$ | $1,09 \pm 1,53$ |
| DK633/266 | 30 | 379 | 0 | $85,49 \pm 3,62$ | $5,54 \pm 2,35$ | $79,95 \pm 4,12$ |
| | 60 | 379 | 0 | $35,88 \pm 4,93$ | $3,17 \pm 1,80$ | $32,72 \pm 4,83$ |
| DK298 | 30 | 360 | 0 | $81,11 \pm 4,13$ | $35,83 \pm 5,06$ | $45,28 \pm 5,25$ |
| | 60 | 360 | 0 | $71,94 \pm 4,74$ | $31,94 \pm 4,92$ | $40,00 \pm 5,17$ |
| DK633 | 30 | 0 | 294 | 0 | 0 | 0 |
| | 60 | 167 | 127 | $22,11 \pm 4,85$ | $13,61 \pm 4,01$ | $8,50 \pm 3,26$ |
| DK3070 | 30 | 0 | 155 | 0 | 0 | 0 |
| | 60 | 96 | 59 | $15,48 \pm 5,83$ | $15,48 \pm 5,83$ | 0 |
| DK236 | 30 | 0 | 429 | 0 | 0 | 0 |
| | 60 | 56 | 373 | $1,40 \pm 1,14$ | 0 | $1,40 \pm 1,14$ |
| DK633/325 | 30 | 0 | 502 | 0 | 0 | 0 |
| | 60 | 336 | 166 | $9,96 \pm 2,68$ | $7,97 \pm 2,42$ | $1,99 \pm 1,25$ |
| Chi31 | 30 | 432 | 0 | $64,81 \pm 4,60$ | $31,53 \pm 3,96$ | $43,29 \pm 4,77$ |
| | 60 | 432 | 0 | $16,90 \pm 3,61$ | $6,94 \pm 2,45$ | $9,95 \pm 2,88$ |

Table 2. Fresh callus weight, frequency of spontaneous regeneration and frequency of brown calli in culture of Lancaster inbreds

| Genotype | DIC | Fresh weight, g | Frequency of | Frequency of brown |
|-----------|-----|---------------------|------------------|--------------------|
| | | | spontaneous | explants, % |
| | | | regeneration % | |
| DK267 | 30 | - | 0 | 0 |
| | 60 | $0,0475 \pm 0,0093$ | 0 | 0 |
| DK212 | 30 | - | 0 | 0 |
| | 60 | $0,0364 \pm 0,0049$ | 0 | 0 |
| DK6080 | 30 | - | 0 | 0 |
| | 60 | $0,1383 \pm 0,0187$ | 0 | 0 |
| DK420-1 | 30 | - | 0 | 0 |
| | 60 | $0,0473 \pm 0,0068$ | 0 | 0 |
| DK633/266 | 30 | - | $15,57 \pm 3,73$ | 0 |
| | 60 | $0,3442 \pm 0,0495$ | $20,32 \pm 4,14$ | 0 |
| DK298 | 30 | - | 0 | 0 |
| | 60 | $0,1500 \pm 0,0156$ | $5,83 \pm 2,47$ | 0 |
| DK633 | 30 | - | 0 | 0 |
| | 60 | $0,0563 \pm 0,0071$ | 0 | $7,82 \pm 3,14$ |
| DK3070 | 30 | - | 0 | 0 |
| | 60 | $0,0704 \pm 0,0090$ | 0 | 0 |
| DK236 | 30 | - | 0 | 0 |
| | 60 | $0,0325 \pm 0,0029$ | 0 | $11,42 \pm 3,07$ |
| DK633/325 | 30 | | 0 | 0 |
| | 60 | $0,0509 \pm 0,0054$ | 0 | $3,78 \pm 1,71$ |
| Chi31 | 30 | _ | 0 | 0 |
| | 60 | $0,1021 \pm 0,0136$ | 0 | 0 |

At 30 DIC inbred DK267, DK 212, DK6080, DK420-1, DK633/266, DK298, Chi31 were suitable for callusogenesis, but the rest ones had appeared only swollen scutellums.

To 30 DIC the morphogenic callus frequency was the highest in DK633/266 and DK298, and the lowest one observed in DK420-1. The frequency of callus type I initiation was the highest in DK267, and the frequency of callus type II initiation was the highest in DK633/266.

To 60 DIC the morphogenic callus frequency was the highest in DK298, and the lowest ones observed in DK6080 and DK420-1. The frequency of callus type I initiation was the highest in DK267, and the frequency of callus type II initiation was the highest in DK298.

Inbred lines, which showed callusogenesis before age 30 days in culture within the period from 30 DIC to 60 DIC, largely decreased the frequency of morphogenic callus initiation. The decrease of frequency of callus type I can be explained by transformation into callus type II or grown brown calli. Callus type II transformated into nomorphogenic callus. It should be noted that inbred DK6080 had the rare events of formation callus type I at 60 DIC. Inbred DK633/266 and DK298 had shown increased frequencies of spontaneous regeneration.

Inbred lines, which showed swollen scutellum before age of 30 days in culture within the from period 30 DIC to 60 DIC, had formed morphogenic callus. The higher ability to morphogenic callus formation was observed in DK633, the lower one – in DK236. It should be noted that some inbred lines had formed calli of both types (DK633, DK633/325), some inbreds had formed only calli of type I (DK3070) or type II (DK236). Inbred lines DK633, DK236 and DK633/325 at 60 DIC had shown grown brown explants. This process was connected with callus age while their callusogenesis ability was appeared only after 30 DIC.

The comparision of callus fresh weights at 60 DIC had shown that DK633/266 had the highest weight, but DK212 and DK236 had the lowest ones.

Thus, the responsive ability of maize immature embryos in culture is strongly genetic depended.

Inbreds DK633/266 and DK298 are recommended to use in future in biotechnological and breeding programmers, because of their ability to formation of morphogenic calli, maintainance of their morphogenic potential during 60 days in culture and quick accumulation of fresh weight.