Leaf disc callus from sugarbeet breeding lines for biolistic transformation

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Introduction

A particle bombardment method for introducing foreign genes into sugarbeet was developed in this lab (Snyder et al., 1999). This method is based on the use of hypocotyls as a source of embryogenic callus for the transformation step. However, this is a lengthy protocol that requires a 3-week seed germination period followed by a hypocotyl cultivation period of 6 to 8 weeks. Seed germination is also often hampered by persistent fungal contamination and hypocotyl isolation is time consuming. Transformation frequencies obtained with embryogenic hypocotyl callus from a noncommercial line, REL-1, were low. Therefore, we explored alternative sources of embryogenic sugarbeet callus for use with the particle bombardment method for sugarbeet transformation.

Materials and Methods

Plants

Sugarbeet line REL-1 (Saunders at al. 1992) and lines C69, C78, Z731, C76-89-5, and 7911-4-10 (Dr. R. Lewellen, ARS, Salinas, CA) and FC607 (Dr. L. Panella, ARS, Ft. Collins, CO) were used in these experiments.

Excision and culture of leaf discs

Leaves from 6 to 12 month old greenhouse-grown plants or from 3 week old seedlings germinated in soil were sterilized in 20% commercial bleach, 0.01% SDS solution for 20 min and washed with sterile water. Leaf discs (d=9 mm) were excised and cultured in the dark on B1 medium (MS basal medium with 1 mg l^{-1} BAP) (Doley and Saunders, 1989) at different temperature regimes indicated in Table 2.

Results and Discussion

Callus forming ability and shoot regeneration on leaf disc explants from different sugarbeet breeding lines

Leaf discs were excised from the youngest, not fully expanded leaves of greenhouse-grown plants and cultured on B1 medium at 30^0C in the dark. All tested sugarbeet lines generated leaf

disc callus, but breeding line FC607 was equal to the REL-1 tissue culture clone in its ability to form callus with shoots at 30°C after 6 weeks of culture (Fig. 1).

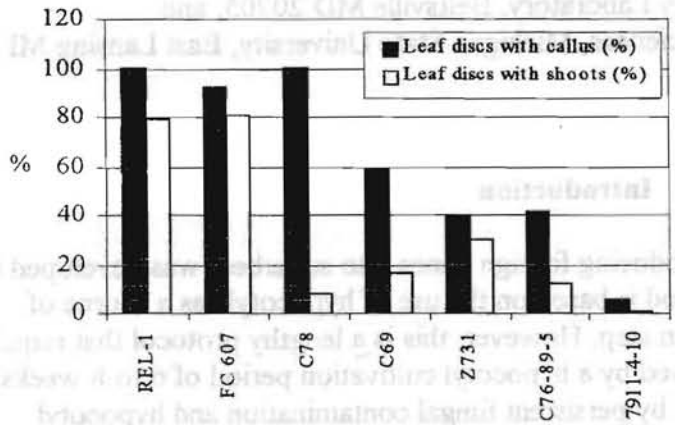

Fig. 1. Callus growth and shoot regeneration on leaf discs from six sugarbeet breeding lines and control line REL-1.

Callus growth and shoot regeneration on leaf discs from FC607 seedlings

Leaf discs were excised from the first pair of leaves of 3-week-old FC607 seedlings and cultured on B1medium at 30°C in the dark. More than 75% of the discs formed friable callus after 6 to 7 weeks of culture. Shoots regenerated at a rate of 29% after 6 weeks in culture. After 7 weeks, the percentage of discs with shoots increased to 57% with an average of 10 shoots/ leaf disc (Table 1).

Table 1. Callusing and shoot regeneration response of leaf dics excised from the FC607 seedlings.

Scored at	Leaf discs with callus (%)	Leaf discs with shoots (%)	Average number of shoots per leaf disc
6 weeks	75	29	2
7 weeks	79	57	10

Influence of temperature on callusing and shoot regeneration

To test the effect of temperature on leaf disc organogenesis, leaf discs from greenhouse-grown FC607 and control REL-1 plants were maintained at 25°C and 30°C for 6 weeks, or cultured at 30°C for 1 or 3 weeks and then transferred to 25°C. Results showed that both callusing and organogenic capacity of leaf discs decreased if they were transferred to 25°C after the initial culturing at 30°C (Table 2). FC 607 leaf discs did not regenerate callus at 25°C even after 8 weeks in culture.

Table 2. Influence of temperature on leaf disc callus growth and shoot regeneration of REL-1 and FC607 sugarbeet lines

Temperature Regime	Discs with callus (%)		Discs with shoots (%)	
	REL-1	FC607	REL-1	FC607
30^0C (6 weeks)	100	60	100	50
30^0C (3 weeks)*	75	42	70	25
30^0C (1 week)*	56	25	40	25
25^0C (6 weeks)	45	0	38	0

* Discs transferred to 25^0C after initial cultivation at 30^0C

Conclusion

Of the tested sugarbeet breeding lines, only callus from FC607 leaf discs showed high shoot regeneration potential that was comparable to the tissue culture clone REL-1. Both mature plants and seedlings of FC607 line proved to be good source of embryogenic callus. The advantages of using leaf disc callus instead of hypocotyl callus with the particle bombardment method include minimal contamination rate (less than 1%) in tissue culture; ease of handling the material; and relatively large quantities of callus that could be generated in a short period of time.

References

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