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Effects of glycerol on *in vitro*-grown *Amaranthus retroflexus* L.

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SUMMARY. Our experiments used a robust plant species (*Amaranthus retroflexus*), that grows on various types of substrate and in a variety of stress conditions (drought, frost, flooding, etc.) to test its survival on culture media with glycerol as a substitute of sucrose. After aseptic seed germination, plantlets were grown on a calus induction culture medium (Gamborg basal medium enriched with NAA and kinetin). The non-morphogenetic calus was cultured on four culture media with ascending glycerol concentration replacing sucrose as a carbon source. The survival and the growing rate of the caluses are factors that lead to the conclusion of succesful degradation of glycerol by *A. retroflexus*. Further biochemical analyses will reveal the biodegradation pathways and the secondary compounds production.

Keywords: *Amaranthus retroflexus*, callus, glycerol, morphogenesis.

Introduction

Glycerol is a secondary product from biodiesel production that is transformed in other useful compounds for polymer industry. The two enzymes that are involved in this process are lipase and decarboxylase.

Amaranthus retroflexus, is an annual species belonging to Amaranthaceae family. It is native to the tropical Americas, but is widespread as an introduced species on most continents in a great number of habitats. The plant is monoecious, with individuals bearing both male and female flowers. The inflorescence is a large, dense cluster of flowers interspersed with spiny green bracts. The fruit is a capsule less than 2 mm long with a "lid" which opens to reveal a tiny black seed (Pammel, 1903). *A. retroflexus* is an annual weed which reproduces only by seeds. It is a prolific seed producer, one single vigorous plant being capable of producing between 230,000 and 500,000 seeds (Stevens, 1957). Seed production is in decline when light is limited

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(MacLachlan *et al.,* 1995). Germination requirements and dormancy patterns are also variable depending on species distribution and local climatic and ecological conditions so, generalizations should be avoided. Recent studies have suggested that germination is stimulated by light intensity and higher levels of temperature (Gallagher and Cardina, 1997; Oryokot *et al*., 1997).

A. retroflexus is an aggressive and competitive weed in a variety of row crops. It causes substantial damages in soyabean, maize, cotton, sugarbeet, sorghum, and many other vegetable crops (Weaver and MacWilliams, 1980). It is edible for animals and has a nutrient composition and digestibility at a level equivalent to that of alfalfa (Marten and Andersen, 1975; Moyer and Hironaka, 1993). Previous studies on *in vitro* multiplication and callus induction of other species of *Amaranthus* were performed with notable results (Flores *et al.,* 1982; Bennici *et al*, 1992). The authors obtained callus starting from hypocotyl and stem tissues but also on leaf disks on *A. cruentus, A. tricolor, A. hypochondriacus, A. caudatus* and *A. hybridus.* Other studies have revealed antioxidant properties and antimicrobial effects on *A. lividus* respectively on *A. spinosus* (Ozsoy *et al*, 2009; Vardhana, 2011).

The main objective of this study was to evaluate the capacity of this species to survive and grow on a culture medium containing glycerol as a carbon source. Further studies will determine the products resulted from glycerol degradation and their use in polymer industry.

Materials and methods

Seeds of *A. retroflexus* were sterilised with succesive washes in 70% ethanol for 30 seconds, 5 minutes with sterile distilled water, 5 minutes with Na hypoclorite 5% and 10 minutes with sterile distilled water. Then, seeds were transfered to (MS) culture medium (Murashige and Skoog 1962). After two weeks of aseptic seed germination, plantlets were grown on a callus induction culture medium B5 (Gamborg basal medium enriched with 0.1 mgL^{-1} NAA and 0.2 mgL^{-1} Kinetin) (Gamborg *et al.,* 1968). The non-morphogenetic calus was grown on four variants of the same medium but with ascending glycerol concentration (from 0 to 30 g L^{-1}) replacing the sucrose as a carbon source (Table 1).

The growing rate was calculated as the weight (g) difference between the weight of callus at the end of experiments (40 days after transfer to media supplemented with glycerol) and the initial weight of callus (at the begining of the experiments). The difference was considered as the callus growth under the influence of glycerol addition in culture medium comparing with control.

In our experiments calluses grown on the four media variants have been tested first for survival and then for growing with a specific rate in 5 repetitions each variant.

Table 1.

Variants of culture media tested

Media/supplement (g* L^{-1})	**1**	**2**	**3**	**4**
Glycerol	0	10	20	30
Sucrose	30	20	10	0

Results and discussion

Our experiments used seeds from *Amaranthus retroflexus*, that grows on various substrate types and in a variety of stress conditions (drought, frost, flooding, etc.) to test its survival on culture media with carbon source replaced by glycerol.

As other authors explained, there is a metabolic pathway in plants that converts glycerol in glucose via gluconeogenesis (chlamypw.mpimp-golm.mpg.de; biologydiscussion.com). In this way, it can be used as a carbon source replacing sucrose in culture media when it is the only source available. For this reason we tested four culture media variants in order to evaluate the capacity of survival and growing rate on a medium with less or without glycerol, forcing the callus to adapt to another carbon source and to swich the metabolic pathway to convert glycerol.

As a control we used a culture medium with 30 g L^{-1} sucrose without addition of glycerol, the rest of culture conditions remaining unchanged. The other three variants were media with a succesive addition of glycerol replacing sucrose in order to reach the same concentration of 30 g* L^{-1}. It is known that glycerol is used in microbiology as an alternative carbon source, replacing sucrose with very good results (Stasiak-Rozanska *et al.,* 2014; Chen *et al.,* 2012).

As it can be observed in Figures 1-4, the calluses survived, after 40 days of cultivation, on all four variants of culture media, keeping the same colour and texture.

The growing rate varies from one medium to another having in general decreasing values with decreasing sucrose concentration and higher levels of glycerol concentrations. As it was expected, the best growing rate was obtained on standard medium without glycerol addition with an increase of 9.98 g (Fig. 1). Other high growing rates were recorded on medium with 10 g* L^{-1} glycerol and 20 g* L^{-1} sucrose with a gain of 7.40 g in the 40 days period (Fig. 2). The increase of callus mass with 6.47 g was recorded on the third medium variant containing only 10 g sucrose and 20 g glycerol (Fig. 3). The most interesting result is the survival and the weight gain on the fourth medium tested where the carbon source was completely replaced by glycerol, forcing the cells to adapt to the alternative metabolic pathway converting glycerol to glucose to have an efficient energy source (Fig. 4).

The survival and the growing rate of the calluses are factors that lead to the conclusion of succesful degradation of glycerol by *A. retroflexus* (Fig. 5). Further biochemical analyses will reveal the biodegradation pathways and the secondary compounds production.

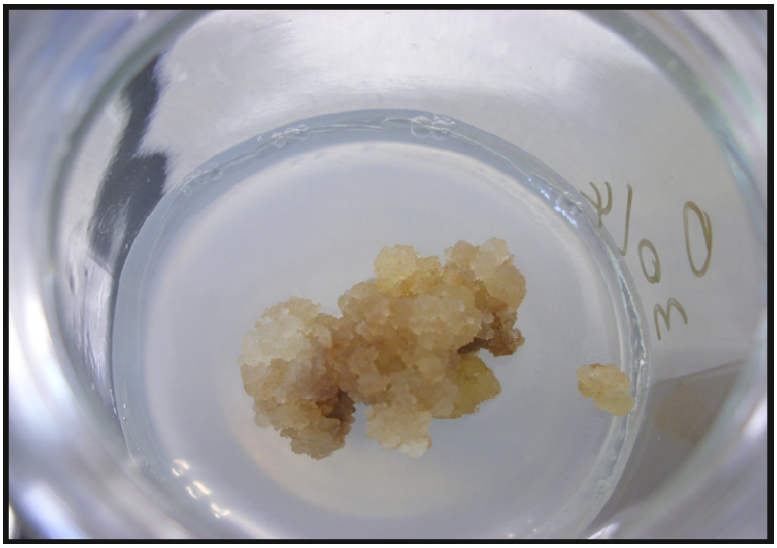

Figure 1. Callus grown on control medium (no glycerol)

Figure 2. Callus grown on culture medium with 10 g* L^{-1} glycerol

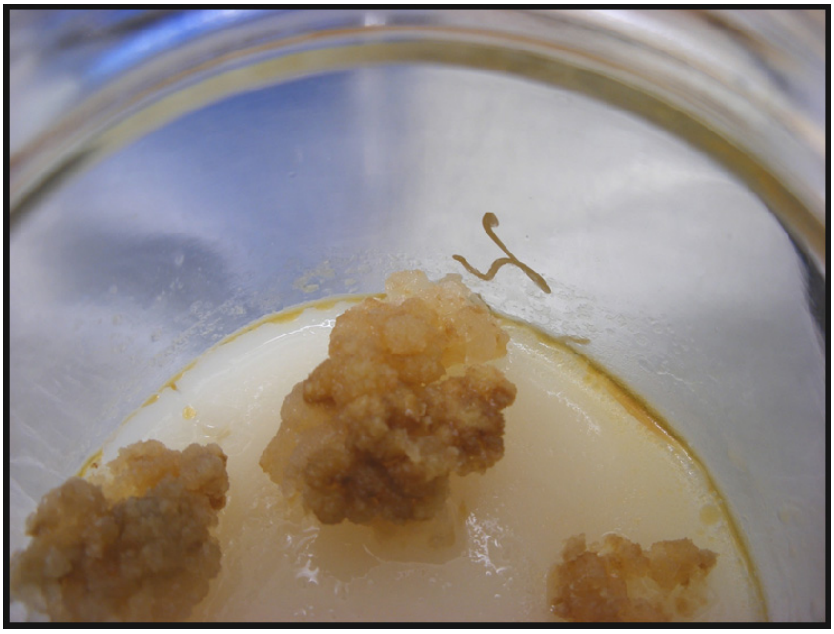

Figure 3. Callus grown on culture medium with 20 g* L^{-1} glycerol

Figure 4. Callus grown on culture medium with 30 g* L^{-1} glycerol

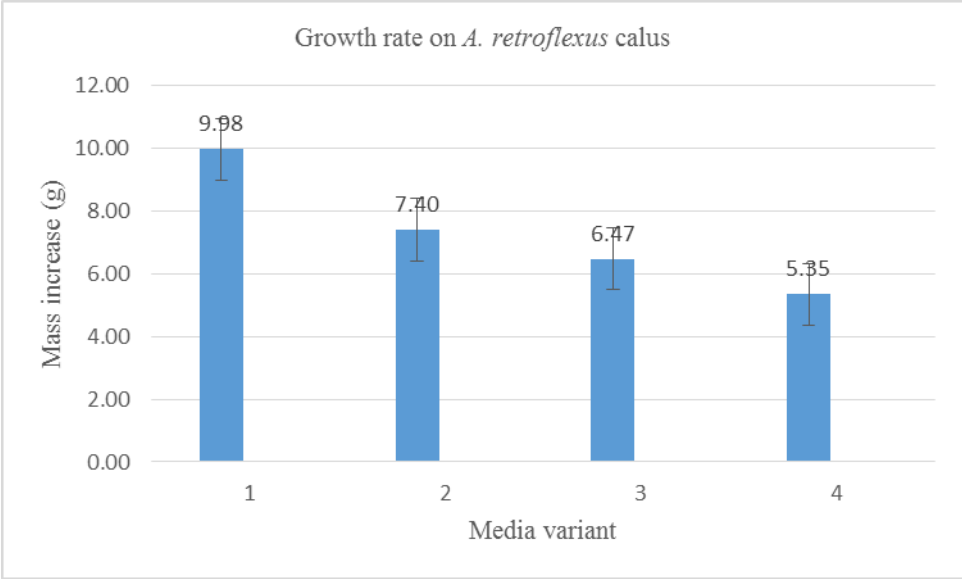

Figure 5. Growth rate of *A. retroflexus* callus on the four essayed media variants.

Conclusions

The results presented above reveal a successful growth rate of callus on culture media with glycerol as an alternative source of carbon replacing sucrose in standard medium. It means that plant cells have switched their metabolic pathway to glycerol degradation to glucose and other similar compounds as energy source. These results may be used in industrial applications and could be a viable solution especially for the polymer industry that produces polyglycerol (one of the sub-products). It offers a solution for consuming the big quantities of produced glycerol affecting the waste waters and the natural habitats. It could be a challenge for plant biotechnologies to help the industry having a positive impact to the environment.

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