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Use of mutation techniques to improve metal uptake in sunflower

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Objectives

Conventional mutation techniques have been successfully used to improve yield, quality, salt tolerance, diseases and pest resistance (1). While oil crops (Helianthus annuus L. and Bras-sica specs.) can accumulate moderate concen-trations of heavy metals from contaminated soil and water (2), their high biomass production makes them interesting for the phytoextraction use. In addition, breeding strategies, such as in vitro-breeding techniques and mutagenesis may help to improve the existing, insufficient effectiveness of metal phytoextraction of these high yielding crop species (3).

The aim of our study (part of PHYTAC) was to apply a non-GMO approach to improve the efficiency of heavy metal uptake in sunflower.

Material and methods

Prior to mutation breeding, heavy metal uptake of 15 commercial sunflower varieties were investigated under real field conditions on a sewage sludge contaminated sandy soil at Rafz (CH) with the following soil properties:

pH 6.8 (H2O) pH 5.8 KCl; Cd 0.9, Zn 1254, Cu 57, Pb 492mg/kg, total content (HNO3, CH) and Cd ‹ 0.01, Zn 5.5, Cu 0.26, Pb 0.04 mg/kg; plant available content 0.1M Na(NO3)2, CH.

Different sources of fertilizer (ammonium & potassium sulphate, ammonium nitrate, potassium chloride) were used, in order to enhance the bioavailability and phytoextrac-tion efficiency of tested sunflowers. After microwave digestions, important metals (Cd, Zn, Pb) were determined by flame AAS.

The sunflower hybrid variety "Salut" and three inbred lines were treated with efficient chemical mutagen EMS (Ethyl methane-sulfonate) for various periods.

A batch of about 9000 mutagenized seeds and controls of these sunflower varieties was sown out directly in our experimental field at Rafz for self-pollination, screening and selectioning of promising mutants (M1 & M2) under real field conditions.

Additionally, accumulation/extraction of Cd, Zn, Pb in mutants and control varieties of sunflower were investigated on modified Hoagland solution spiked with 10µM Cd, 10oµM Zn and 10µM Pb.

Acknowledgment

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Results

Field-based screening of 15 commercial sunflower varieties for metal accumulation and extraction showed a natural variation of a factor 2-3 for important metals (Cd, Zn, Pb) between individual sunflower varieties (Fig. 1). Another factor of 2 in metal uptake was observed in the plants of the same variety, but treated with various sources of fertilizer, such as ammonium-sulfate or ammonium-nitrate. Ammonium-sulfate seems to be an environmental friendly „mobilisation" treat-ment for enhancing the extraction of lead, zinc and particular cadmium in

Mutagenesis of sunflower

According to this field screening, sunflower hybrid "Salut" was chosen as the most promising variety for mutation breeding.

In addition, seeds of three inbred lines (IBL04, 05, 06) were also treated with efficient chemical mutagen EMS, which

Fig. 2: Healthy and flowering mutant of inbred line 04.

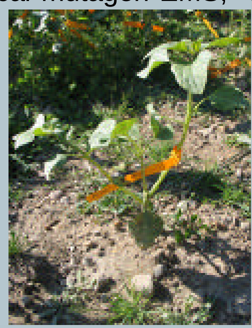

Fig. 3: Mutant of inbred line 04 with injury in branch structure.

Find out best sunflower for mutagenesis

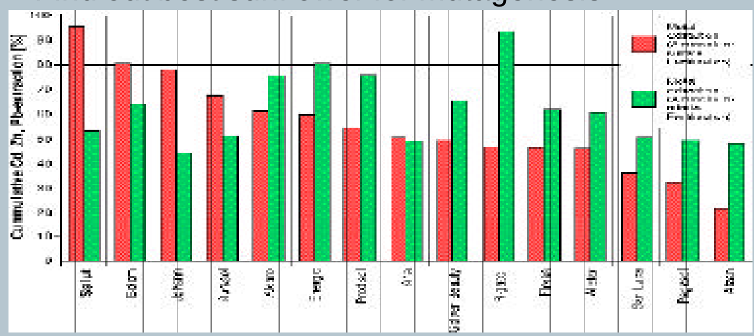

Fig. 1: Sunflower hybrid „Salut" was the winner in combined Cd, Zn Pb extraction at Rafz and selected for mutagenesis.

causes high frequency of mutations in sunflower. The mutation effect of EMS on the germinability, survival capacity, chlorophyll and growth abberations, biomass production and heavy metal accumuation and extraction of sunflower was observed in the laboratory and in the field.

The main goal of the experiment was to test sunflower mutants under real field conditions at the sewage sludge contaminated site of Rafz, in order to find out the most accumulating mutants of sunflower (Fig 2-4).

Fig. 4: Mutant of inbred line 06 with several symptoms of dwarfism

Self pollination of mutagenised sunflower for screening in M2-generation

The most promising fertile mutants of sunflower M1 genera-tion were selfed (Fig. 5) with the aim, to obtain sufficient amount of seeds of M2 generation. Due to the extreme drought in summer 2003 a second sowing became necessary.

A subsequent field-based screening of M2 generation on the Rafz site in 2004 will allow to find out recessive mutants

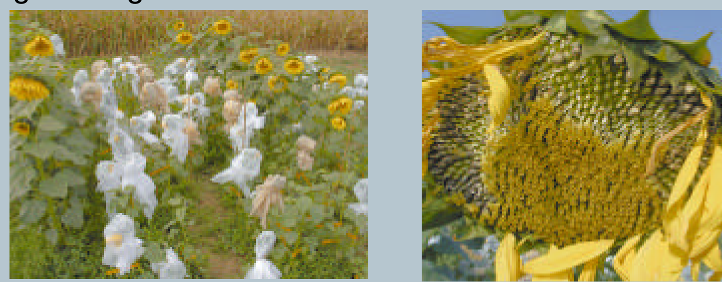

Fig. 5: Self-pollination of M1 sunflowers at the Rafz site (CH) for mutant screening in M2.

Testing of mutagenised sunflower on hydroponics

The results of hydroponic experiment show the effect of EMS on metal accumulation of mutagenised sunflower. Among the mutagenised sunflowers accumulator and excluder types were found and the metal content of mutants varies by a factor 3 – 240, depending of the sunflower variety and the metal under consideration.

The best gain in Cd, Zn, Pb shoot accumulation are observed in variety „Salut" that was treated with EMS for a longer period of time, compared with inbred lines (Fig. 6). The increased duration of mutagenesis generally causes higher frequency of mutations, but often decreases the biomass production. Mutagenised inbred line 04 also combines promising metal accumulating mutants with a good growth, that leads to a gain in metal extraction (Fig. 7).

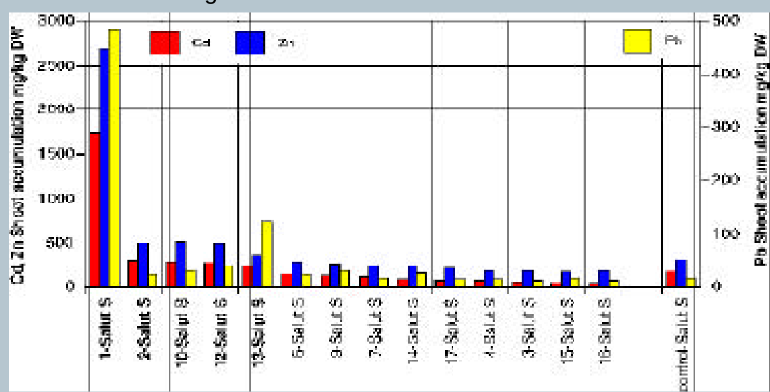

Fig. 6: Shoot accumulation of „Salut" mutants. Individual mutant „1-Salut S" shows an inhanced Cd, Zn, Pb uptake of a factor 9-30, compared with controls. Mutants with enhanced uptake are written in bold.

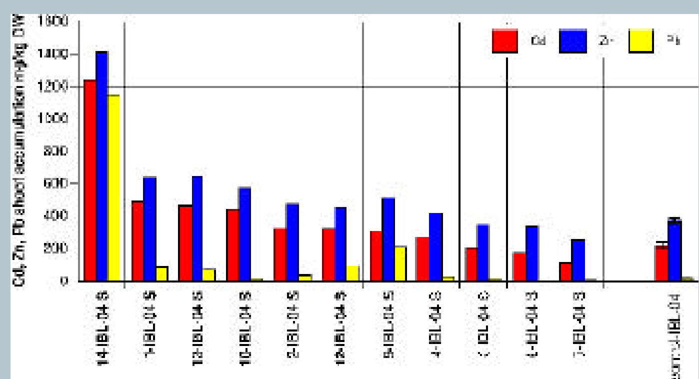

Fig. 7: Mutant 14-IBL04 of inbred line IBL04 shows a gain in shoot uptake (in bold) of a factor 4-6 for Zn, Cd and Pb, respectively. Mutants with enhanced uptake are written in bold.

Conclusions and outlook

As can be seen from the hydroponic experiment, EMS muta-genesis leads to an enhanced shoot accumulation and extrac-tion in sunflower. Among the putative mutants, individual vari-ants with enhanced Cd and/or Zn, Pb accumulation were already found in M1 generation, representing dominant mutants.

However, the effect of mutagens on metal uptake and extrac-tion of sunflower variants in M1 generation was mainly studied at the metal contaminated Rafz site, in order to obtain sufficient amount of selfed seeds for a more detailed screen-ing of recessive mutants of M2 plants in 2004, and to assess the putative mutans under real field conditions.

Preliminary analyses of the metal content of sunflower oil, produced on metal contaminated soil, indicate a good chance for an additional use for technical purposes, such as lubricants or renewable energy. This would reduce the

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