CONCENTATION OF MINERAL ELEMENTS IN CALLUS TISSUE CULTURE OF SOME SUNFLOWER INBRED LINES

M. Saric[1], Dragana Vasic, Lj. Vasiljevic, D. Skoric, Snezana Mezei and Slobodanka Pajevic[2]

ABSTRACT

Concentration of mineral elements in calli of six sunflower inbred lines have been determined. Calli were induced from cotyledons of seven days old seedlings grown under sterile conditions. The differences between inbred lines in concentration of N, P, K, Mg, Fe and Mn were significant, whereas differences in the concentration of Ca, Na, Cu and Zn were insignificant. Average concentrations of mineral elements in calli were lower than average concentrations of mineral elements in leaves of plants grown *in vivo*.

Key words: mineral elements, callus, sunflower, inbred lines.

INTRODUCTION

Numerous criteria have been used to evaluate the obtained effects of genetic specificity of the mineral nutrition in plants. It should be emphasized that in previous investigations the concentartion and content of mineral elements in plants have been most frequently used as criterion for the occurrence of genetic specificity. Cultivar specificity of mineral nutritions is manifested through the differences in ion uptake, their transport, distribution and accumulation in individual organs, as well as in their reutilization, efflux, metabolic role and biomass production. The differences mentioned are found in plant organs of different age of both cultivated plants and those inhabiting natural phytocenoses. All differences are found under both artificial and natural conditions. Numerous results, cited in reviews (Epstein, 1972; Saric, 1981, 1983; Clark, 1983), show that there is a difference between genotypes of different plant species concerning concentration of some elements of mineral nutrition of plants grown *in vivo*. A review of results concerning sunflower (*Helianthus annuus* L.) is given the paper of Saric et al. (1991).

Problems of genetic specificity of mineral nutrition of plants grown *in vitro* were investigated to a lesser extent. One must keep in mind the fact that physiology and biochemistry of plants grown *in vitro* differ from those grown *in vivo*. Here is one example. The intensity of growth and development of plants grown *in vivo* is a function of net photosynthesis which, most often, correlates with the intensity of photosynthesis and the leaf area. *In vitro*, the growth is a function of mineral elements and organic components of the medium, because the intensity of photosynthesis and leaf area are negligible.

Physiological and biochemical differences are fully expressed in callus cultures. The growth and morphogenesis of plants grown *in vitro* could be effectively influenced by optimal concentration of the elements of mineral nutrition and other organic components. The success of growing plants *in vitro* is related primarily to the composition of the medium.

Still, the number of papers dealing with problems of genetic specificity of mineral nutrition of plants grown *in vitro* is considerably sparse. The review of papers dealing with genetical aspect of mineral nutrition of plants grown *in vitro* is given in paper of Saric et al. (1995).

We believe that the results referring to mineral nutrition of plants grown *in vitro* will contribute not only to improving this method, but also to the further progress in solving the problems of genetic specificity of mineral nutrition of plants and even the ion uptake mechanism.

MATERIALS AND METHODS

Six sunflower inbred lines, Ha-74A, PH-BC_2-152A, Ha-26A, L-1A, OCMS-48 and RHA-SNRF were used in the experiment. Each inbred line was represented by ten

Institute of Field and Vegetable Crops, M.Gorkog 30, 21000 Novi Sad, Yugoslavia
[1] Serbian Academy of Sciences and Arts, Knez Mihajlova 35, 11000 Belgrade, Yugoslavia
[2] Faculty of Natural Sciences, Trg Dositeja Obradovica 7, 21000 Novi Sad, Yugoslavia

plants, which were donors of the initial explants.

Calli were induced from cotyledons, taken from seven days old sunflower seedlings grown in a sterile environment (Vasiljevic and Vasic, 1994). The explants were cultured on the MS medium (Murashige and Skoog, 1962), supplemented with 1 mll^{-1} benzylaminopurine and 2 mll^{-1} naphtaleneacetic acid.

After three weeks of culture calli were cut in two parts. Mass of fresh and dry matter of one part of each callus was measured. The other part of each callus was placed on fresh medium and grown under fully controlled temperature (25^0C), photoperiod (16:8; light:dark) and humidity (85%). The same procedure has been repeated for five times. After that, calli were rinsed with distilled water, dried to constant air-dry mass, macerated and prepared for chemical analyses.

Calli were analysed for nitrogen content by the Kjeldal method, phosphorus content by molybdate-vanadate spectrophotometry, potassium content by flame photometry, calcium, manganese, sodium, iron, zinc, magnesium and copper content by atomic absorption spectroscopy.

Data were processed by the analysis of variance.

RESULTS AND DISCUSSIONS

The differences in the concentration of mineral elements in the calli obtained from different inbred lines varied from highly significant to insignificant (Tables 1 and 2). Avrage concentration of mineral elements in calli were compared to average concentrations of mineral elements in root, stem and leaves of intact plants (Saric et al., 1991) (Figures 1, 2 and 3).

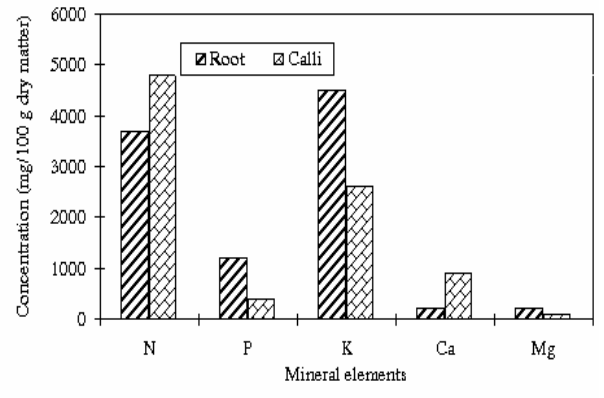

Figure 1. Average concentrations of some mineral elements in calli and root of sunflower inbreds

Table 1. Concentration of some macroelemts in calli of six sunflower inbreds (mg/100 g dry matter)

Genotype	N***		P**		K*		Ca^{ns}		Na^{ns}		Mg***	
Ha-74A	3197	c	281	b	2833	a	825	a	700	a	96	a
PH-BC_2-152A	4669	b	331	ab	2417	a	808	a	767	a	89	bc
Ha-26A	5222	a	350	a	1917	a	841	a	750	a	90	bc
L-1A	5534	a	362	a	2783	a	725	a	767	a	87	c
OCMS-48	5282	a	315	ab	2583	a	816	a	767	a	91	b
RHA-SNRF	4828	b	332	ab	2833	a	733	a	650	a	99	bc
Average	4789		328		2561		792		733		90	
LSD 5%	218		31		511		180		89		1.7	
1%	306		44		716		282		125		2.4	

Table 2. Concentration of some microelemnts in calli of six sunflower inbreds (ppm)

Genotype	Fe***		Mn***		Zn^{ns}		Cu^{ns}	
Ha-74A	225	b	212	a	167	a	18	a
PH-BC_2-152A	252	b	160	c	148	a	17	a
Ha-26A	278	ab	178	bc	165	a	20	a
L-1A	252	b	160	c	190	a	13	a
OCMS-48	285	ab	180	bc	198	a	17	a
RHA-SNRF	315	a	193	b	192	a	15	a
Average	268		180		177		17	
LSD 5%	32		12		36		5.5	
1%	45		17		51		7.8	

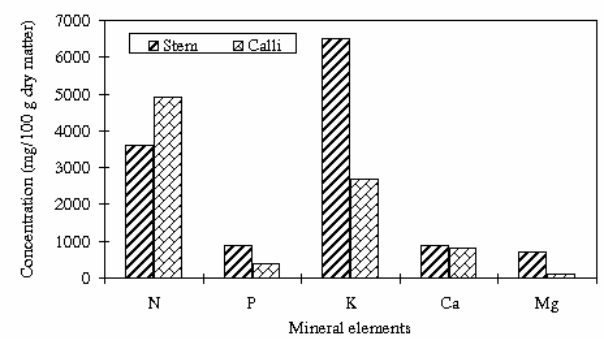

Figure 2. Average concentrations of some mineral elements in calli and stem of sunflower inbreds

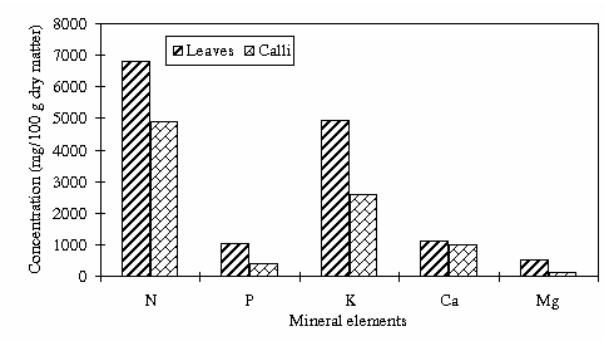

Figure 3. Average concentrations of some mineral elements in calli and leaves of sunflower inbreds

Nitrogen

Nitrogen concentration in calli ranged from 5534 (L-1A) to 3197 mg/100 g of dry matter (Ha-74A). Average nitrogen concentration of all six inbred lines was 4789 mg/100 g of dry matter. Average nitrogen content of twenty sunflower inbreds grown *in vivo* depending of plant organ was 6765 (leaves), 3462 (stem) and 3829 (root) mg/100 g of dry matter. On the basis of the obtained results it could be concluded that nitrogen concentration in calli of inbred lines was within the limits of plants grown *in vivo.*

Phosphorus

Phosphorus content varied as well, and the order of minimal and maximal concentrations was the same as for nitrogen, i.e. Ha-74A had the lowest (281 mg/100 g of dry matter) and L-1A the highest phosphorus concentration (362 mg/100 g of dry matter). In comparison to phosphorus concentration in intact plants, the average phosphorus concentration was significantly lower in calli (328 mg/100 g of dry matter), because it ranged from 730 to 1687 mg/100 g of dry matter in leaves of intact plants.

Potassium

Potassium concentration was the highest in Ha-74A(2833 mg/100g of dry matter), opposite to nitragen and phosphorus concentration. The lowest concentration was 1917 mg/100 g of dry matter in Ha-26A. In comparison to intact plants where concentration varied from 3900 to 6300 mg/100 g of dry matter it could be said that potassium concentration was more than three times higher in intact plants than in calli.

Calcium

The differences in calcium concentration in calli of tested inbred lines were insignificant. If we compare calcium concentration in *in vitro* and *in vivo* grown plants, calcium concentration in calli was significantly lower than in intact plants.

Sodium

The differences in sodium concentration in different inbreds were insignificant.

Magnesium

The inbred lines tested differ significantly in magnesium concentration. Inbred line L-1A had the highest (96 mg/100 g of dry matter) and PH-BC_2-152A the lowest magnesium concentration (87 mg/100 g dry matter). Magnesium concentration in plants grown *in vivo* ranged from 563 to 822 mg/100 g dry matter while it was five times lower in calli.

Iron

Iron concentration was significantly different in different inbreds and Ha-74A had the lowest (225 ppm) and RHA-SNRF the highest iron concentration (315 ppm)

Manganese

The differences in manganese concentration in tested inbred lines were significant Ha-74A had the highest (212 ppm) and PH-BC_2-152A the lowest manganese concentration (160 ppm).

The differences in the concentration of zinc as well as copper were insignificant.

Calli could be considered convenient for studying the genetic specificity of mineral nutrition because many factors that cause significant variability of results *in vivo* are negligible *in vitro*.

A callus is generally more homogenous than an intact plant, although it is not ideally homogeneous. Its diversity is manifested in the total number of cells, number of meristematic cells, cell size, differentiation of somatic cells, and vacuole size. Division activity of cells is not uniformly arranged throughout callus tissue. It is well known that in *in vitro* culture a new variability can appear in significantly higher percentage than spontaneous mutations. This variability is called somaclonal variability and can be observed on morphological, cytological and molecular level. In our experiment, we wanted to achieve the highest possible genetic uniformity within each tested genotype and to avoid differences in nutrient concentrations which could occur due to genetic divergence within the tested genotypes, so we have used different inbred lines as donors of explants. Differences in concentration of mineral elements were obtained between different sugarbeet cultivars (Mezei et al., 1995).

Average concentration of mineral elements in calli was lower than in intact plants although they were grown on medium supplemented with higher concentrations of nutrients than intact plants. It could be explained by the fact that relative to their total fresh or dry weight, rooted plants *in vivo* have, through root hairs and mycorrizal fungi, a very large surface area involved in nutrient uptake (Mengel, 1984) compared with calli. Also, in *in vivo* nutrient concentrations in medium are kept constat whereas in *in vitro* medium the initial concentrations of nutrients are very high, but then decrease rapidly (Leifert et al., 1995).

Average concentrations of mineral elements in calli have been compared to average concentrations of mineral elements in root, stem and leaves of intact plants. Generally, if we compare concentrations of tested mineral elements in intact plants and in calli, we can conclude that concentration of all mineral elements was lower in calli than in intact plants (Saric et al., 1991), although calli were grown on medium supplemented with higher concentrations of mineral elements than intact plants (Saric et al., 1995). It would be interesting to investigate the reactions of plants of the same genotype but of different origin to individual mineral elements.

REFERENCES

Clark, R.B., 1983. Plant genotype differences in the uptake, translocation, accumulation and use of mineral elements required for plant growth. In: Genetic Aspects of Plant Nutrition. Martins Nijhoff/J.W. Junk Publishers, the Hague/Boston/Lancaster: 49-70

Epstein, E., 1972. Physiological genetics of plant nutrition. In: Mineral Nutrition of Plants: Principles and Perspectives. John Wiley and Sons, New York: 325-344.

Leifert, C., Murphy, K.P., Lumsden, P., 1995. Mineral and carbohydrate nutrition of plant cell and tissue cultures. Crit.Rev.Plant Sci. 14(2): 83-109.

Mengel, K., 1984. Ernahrung und Stoffwechsel der Pflanze. Gustav Fischer Verlag. Stuttgart, Germany.

Mezei, S., Saric, M., Pajevic, S., Kovacev, L., 1995. Concentration of mineral elements in callus tissue cultures of two sugarbeet (*Beta vulgaris* L.) cultivars. Arch.Biol.Sci., Belgrade, 47 (1-2): 29-32.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant 15: 473-497.

Saric, M., 1981. Genetic specificity in relation to plant mineral nutrition. J. Plant Nutr. 3: 743-764.

Saric, M., 1983. Genetic improvement of crops yields as related to plant nutrient requirements. 9th CIEC World Fertilizer Congress. Budapest, Hungary: 115-128.

Saric, M., Krstic, B., Skoric, D., 1991. Element diversity in sunflower inbred lines. Helia 14 (15): 41-48.

Saric, M., Mezei, S., Ruzic, D., 1995. Genetic aspects of mineral nutrition of plants grown *in vitro*. Arch.Biol.Sci., Belgrade 47(1-2): 1-12.

Vasiljevic, Lj., Vasic, D., 1994. Plant regeneration of sunflower (*Helianthus annuus* L.) *in vitro*. Proc. Oil and Protein Crops Meeting of EUCARPIA. Albena, Bulgaria: 182-184.

Table 1. Concentration of some macroelemts in calli of six sunflower inbreds (mg/100 g dry matter)

Genotype	N***		P***		K*		Ca^{ns}		Na^{ns}		Mg***	
Ha-74A	3197	c	281	b	2833	a	825	a	700	a	96	a
PH-BC$_2$-152A	4669	b	331	ab	2417	a	808	a	767	a	89	bc
Ha-26A	5222	a	350	a	1917	a	841	a	750	a	90	bc
L-1A	5534	a	362	a	2783	a	725	a	767	a	87	c
OCMS-48	5282	a	315	ab	2583	a	816	a	767	a	91	b
RHA-SNRF	4828	b	332	ab	2833	a	733	a	650	a	99	bc
Average	4789		328		2561		792		733		90	
5% LSD	218		31		511		180		89		1.7	
1%	306		44		716		282		125		2.4	

Table 2. Concentration of some microelemnts in calli of six sunflower inbreds (ppm)

Genotype	Fe***		Mn***		Zn^{ns}		Cu^{ns}	
Ha-74A	225	b	212	a	167	a	18	a
PH-BC$_2$-152A	252	b	160	c	148	a	17	a
Ha-26A	278	ab	178	bc	165	a	20	a
L-1A	252	b	160	c	190	a	13	a
OCMS-48	285	ab	180	bc	198	a	17	a
RHA-SNRF	315	a	193	b	192	a	15	a
Average	268		180		177		17	
5% LSD	32		12		36		5.5	
1%	45		17		51		7.8	

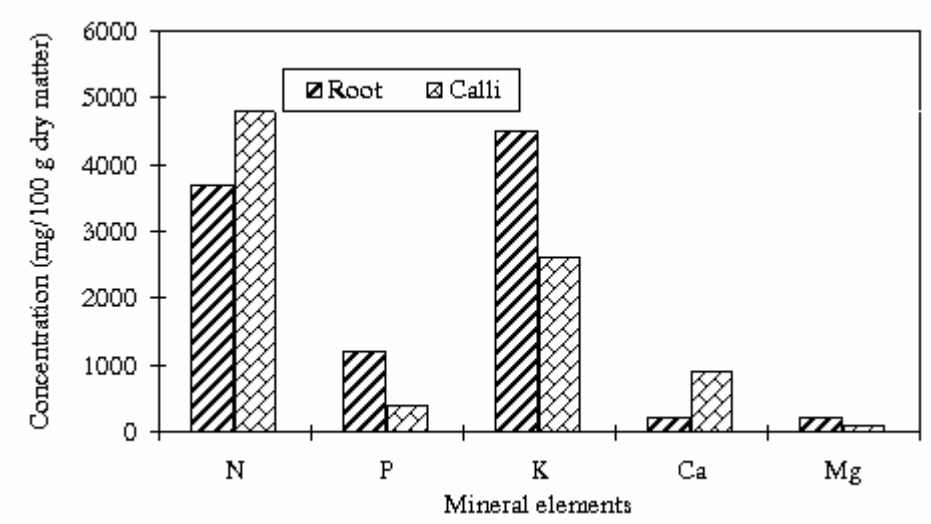

Figure 1. Average concentrations of some mineral elements in calli and root of sunflower inbreds

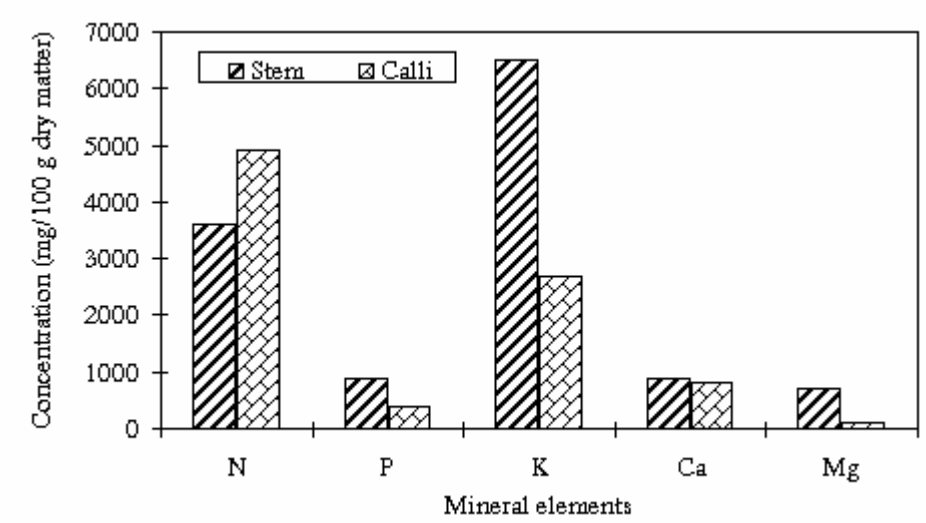

Figure 2. Average concentrations of some mineral elements in calli and stem of sunflower inbreds

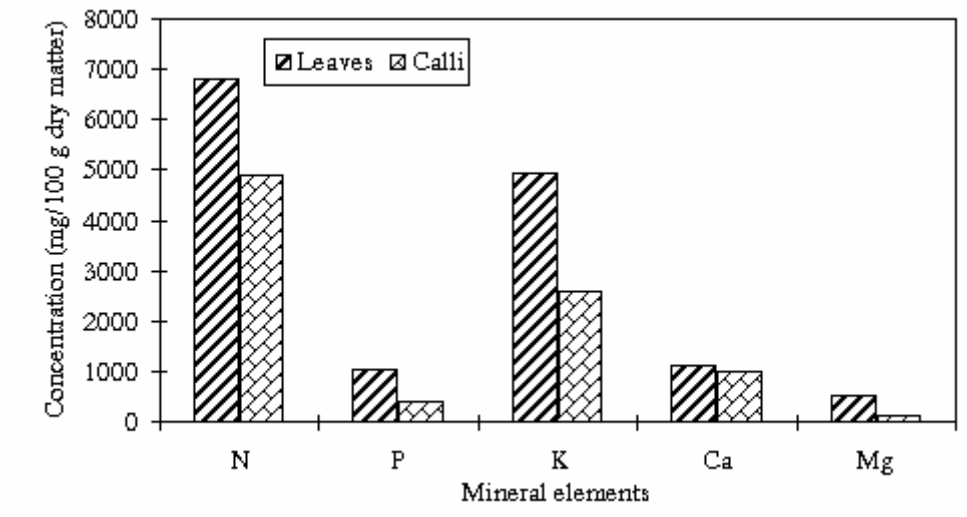

Figure 3. Average concentrations of some mineral elements in calli and leaves of sunflower inbreds