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Development of Plant Model to Study Biological Effects of Nanodilutions

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Summary: *Pea* (*Pisum sativum*) as a model plant has been used extensively in fundamental research in different biological sciences. *In vivo* and *in vitro* pea models were used, as well, to study stress factors. Applying environment friendly technologies for overcoming biotic/abiotic stress increases its importance for sustainable agriculture. In this respect studies in the field of nanotechnology can contribute to solve some problems and to understanding of phenomena or practices that still lack methodology or specific instrumentation for scientific explanations. The interest to such studies was provoked by attempting an explanation on the potentization process and its therapeutic effect, and also by the possibility to apply similar approach in sustainable agriculture. The objectives of the experiments were to examine if potentized nanodilutions (PNDs) have effects on different stages of seed development of pea aiming at the development of a plant model. Copper was chosen as stress factor as its excess is toxic and affects seed development. The experiments show for the first time that potentized nanodilutions (PNDs) of metallic copprer have biological effects on pea seed development which are similar to the effect of copper (water solutions of $CuSO_4$). The results, also, show that PNDs can stimulate response for overcoming the stress applied to seeds.

Keywords: Plant Model, Pea, Nanodilutions, Potentized Solutions, Cuprum, Heavy Metals, Abiotic Stress.

1. INTRODUCTION

Pisum sativum as a model plant has been used extensively in fundamental research in genetics, plant physiology and biochemistry. Pea, unlike other model plants (e.g. *Arabidopsis*) is, as well, an economically important crop for food and nonfood industry, and for sustainable agriculture. Lately one of the biggest problems facing agriculture is overcoming crop damages of biotic and abiotic stress and applying environment friendly technologies. In this respect *in vivo* and *in vitro* research using plant and cell models to study the effect of various stress factors and protectors was recently carried by Kosturkova [1-4]. Based on our experience in genetics, physiology, plant breeding, biotechnology and nanomedicine we proceed further towards the sphere of nanotechnology in order to give us viable solutions to the above stated problems. In this respect studies in the field of nanotechnology/nanomedicine can contribute to understanding of phenomena or practices that still lack methodology or specific instrumentation for scientific explanations.

Present research refers to the biological effects of potentized nanodilutions (PNDs). These are in concentrations of 10^{-12} mole and lower, even beyond the reciprocal of Avogadro's number $6.02x10^{-23}$. Our interest to such studies was provoked by Delinick's hypothesis [5] using biophysics theories in attempting an explanation on the potentization process and therapeutic effect of homeopatic remedies and the possibility to apply similar approach in sustainable agriculture. This is an ongoing challenge to the authors to extend their experience in the field of stress of two different eukariotic systems - plants and humans.

The objectives of the experiments were to examine if PNDs have effects on different stages of seed development of pea aiming at the development of a plant model. Among the numerous stress factors, copper was chosen as its excess is toxic and affects root functions which could be detected at the first stage of seed development – seed germination. However, copper as a microelement participates in various metabolic processes as an essential ligand for many enzymatic catalytic activities and deviations from homeostasis could be observed at different levels of plant development [6].

2. MATERIALS AND METHODS

A. Preparation of the potentized nanodilutions

Potentized nanodilutions (PNDs) were prepared by Delinick [5] according to the instructions given in the German Pharmacopeia [7]. In these experiments metallic copper (Cu-m) was used in several dilutions: 10^{-12}, 10^{-24}, 10^{-60}, 10^{-400}, $10^{-2\,000}$, $10^{-20\,000}$, and $10^{-100\,000}$. Two groups (A and B) of PNDs of metallic copper were used. The A group was made with 99 ml of double distilled water and the B group was made with 99 ml of double distilled water and ethanol.

B. Preparation of the plant material

Seeds of pea (*Pisum sativum*) cv. "Balet" were germinated in Petri dishes (15 cm or 12 cm in diameter) on soft paper tissue and water or the relevant solution was added (60 ml or 25 ml, respectively). Seeds were cultivated in light with photoperiod 16/8h. Copper sulphate solutions (10^{-2}, 10^{-3}, 10^{-4} mol) were prepared from $CuSO_4.5H_2O$ dissolved in distilled water. One drop of PNDs was added to the Petri dish containing water or solution of copper sulphate (10^{-2} mol) before plating the seeds in the Petri dish or after this in different intervals (1h, 4h, 24h, 48h). Different criteria were used to observe the effect on seed development: seed germination, root and stem growth and weight, formation of root branches in different periods.

Thirty to fifty seeds were used per variant.

3. RESULTS AND DISCUSSIONS

Seed germination in water (control) was observed on the 2nd day, as being 50% and reaching 80 % on the third day (Table 1). Copper sulphate suppressed seed germination and root growth depending on its concentration. The highest one 10^{-2} mol $CuSO_4$ arrested root formation on the 2nd day and germination reached only 30% by the 3rd day. Root growth was slower at 10^{-4} mol and very poor at the other concentrations of copper sulphate. Elongation of the root was terminated after the 3rd day and degradation was observed for 10^{-2} mol. At the lowest concentration roots formed branches which were longer than that of the control, what can be explained by the suppression of the apical root dominance.

Table 1. Effect of copper sulphate on root formation and growth

N	Concentration of $CuSO_4$	Germination [%] on day		Root size [cm] on day				Root branches on 7th day	
		2nd	3rd	3rd	4th	6th	7th	[%]	No
1	0 mol, H_2O	50	80	1.4	2.3	5.7	6.9	90	5.3
2	10^{-4} mol	37	70	1.0	1.8	2.1	2.3	80	6.9
3	10^{-3} mol	27	60	0.7	0.7	0.7	0.7	0	0
4	10^{-2} mol	0	30	0.2	0.3	0.8	0.4	0	0

Stem formation was not observed at 10^{-2} mol and was retarded by cuprum sulfate more expressed in 10^{-3} mol. The lowest concentration had no significant effect on stem growth (Table 2).

Table 2. Effect of copper sulphate on stem formation and growth

N	Concentration of $CuSO_4$	Stem formation [%] on day		Stem size [cm] on day			
		3rd	4th	3rd	4th	6th	7th
1	0 mol, H_2O	27	40	0.50	0.78	2.3	2.7
2	10^{-4} mol	23	47	0.34	0.85	2.5	2.7
3	10^{-3} mol	6.7	13	0.35	0.75	1.2	1.5

In another set of experiments similar negative effect on seed development was observed after potentized nanodilutions (PNDs) of metallic copper (Cu-m) were added as a drop to the water where seeds were germinated (Table 3). Differences from the control were

more pronounced in experiments (B) where Cu-m was potentized in water and ethanol. Increasing the dilutions 10^{-2000} to 10^{-20000} and $10^{-100000}$ growth was reduced to 91%, 63% and 41%, respectively of the root length in the control and 94%, 77%, and 68%, respectively, of the stem length in the control.

Table 3. Developmental characteristics of pea seedlings 7 days after addition of a drop of potentized nanodilutions of cuprum metallicum

Variant of PNDs of metallic copper (Cu-m)	Root L	Stem L	Root Wg	Stem Wg	Root branches length [cm]	
	[cm]	[cm]	[mg]	[mg]	min	max
H_2O (control)	9.3	4.7	340	480	1.0	4.0
$10^{-2\,000}$ (A)	9.3	5.1	280	360*	1.9	4.8
$10^{-20\,000}$ (A)	10.6	4.7	220*	286*	1.2	3.6
$10^{-100\,000}$ (A)	11.6*	5.4*	234*	371*	1.6	6.8
$10^{-2\,000}$ (B)	8.5	4.4	335	419*	0.9	3.0
$10^{-20\,00}$ (B)	5.9*	3.6*	174*	313*	1.1	2.1
$10^{-100\,000}$ (B)	3.8*	3.2*	192*	275*	1.0	1.0

Legend: (A) dissolved in double distilled water; (B) dissolved in double distilled water and ethanol; L – length; Wg – weight; * Statistically significant at $P < 0.05$.

Similarly weight was lower ranging for roots between 87% and 56% relative to control and between 87% and 57% for the stem, respectively. Root branching was 100% in all variants with exception of $10^{-20\,000}$ (B) and $10^{-100\,000}$ (B) where it was 43%, however, a tendency of bigger size of the branches was observed in experiments (A).

To avoid speculations about the effect of the solvent/PND of metallic copper an experiment was set up where a drop of the potentized water (variant A) was added to the water for seed germination. This was also done for variant B diluent, where a drop of potentized water and ethanol were added to the water for seed germination. No significant differences were recorded in the development of seeds as presented in Table 4.

Table 4. Development of pea seeds after treatment with a drop of the potentized solvent (A) and (B)

Variant for seed germination	Root length		Root weight	Stem length	Stem weight
	1st day [cm]	3rd day [cm]	3rd day [mg]	3rd day [cm]	3rd day [mg]
H_2O, control	1.14	3.55	61.4	0.72	42.3
H_2O + H_2O	1.41	3.39	67.6	0.85	37.6
H_2O + (A)	1.36	3.71	71.6	0.86	38.9
H_2O + C_2H_5OH	0.98	3.59	61.6	0.78	32.0
H_2O + (B)	1.01	3.37	68.6	0.78	33.1

Legend: (A) potentized double distilled water; (B) potentized double distilled water and ethanol.

In another set of experiments (Table 5 and 6) seeds were subjected to stress being germinated in 10^{-2} mol copper sulphate solution - a concentration which suppressed significantly seed germination and development as was shown in Table 1. Here, germination was reduced twice and root development was very poor representing only 17% of the control. When a drop of PNDs was added the level of suppression was changed. For germination it was reduced by 25-45% in 2 variants (3 and 6) and enhanced by 15-50% in 5 variants (4, 5, 7, 8, 9). Stress relieving effects were bigger for root growth - root

length bigger from 38% to 46% (var. 3, 4, 5, 6, 7, 8) and respectively weight from 35% to 200% (var. 4, 5, 6, 7, 8, 9). Similarly the stress effect of cuprum sulphate was reduced by 25% for stem growth (var 7) and by 13%-64% for stem weight (var 3, 4, 6, 7, 8, 9).

Table 5. Development of pea seeds on the 5th day after treatment with a drop of different PNDs of metallic copper (Cu-m)

N	Variant for seed germination	Germination [%]	Root length [cm]	Root weight [mg]
1	H_2O, control	100	6.0 ± 0.2	165 ± 0.6
2	10^{-2} mol $CuSO_4$	50	0.58 ± 0.07	28 ± 0.77
3	10^{-2} mol $CuSO_4$ + $10^{-20\,000}$ Cu-m on 0 h	72	0.66 ± 0.06*	23 ± 0.58
4	10^{-2} mol $CuSO_4$ + $10^{-2\,000}$ Cu-m on 0 h	43	0.85 ± 0.12*	62 ± 0.62
5	10^{-2} mol $CuSO_4$ + 10^{-400} Cu-m on 0 h	30	0.73 ± 0.14	47 ± 1.0
6	10^{-2} mol $CuSO_4$ + 10^{-60} Cu-m on 0 h	63	0.69 ± 0.09	35 ± 0.62
7	10^{-2} mol $CuSO_4$ + 10^{-60} Cu-m (S)	42	0.75 ± 0.1*	37 ± 0.52
8	10^{-2} mol $CuSO_4$ + 10^{-24} Cu-m (S)	23	0.84 ± 0.19	33 ± 0.60
9	10^{-2} mol $CuSO_4$ + 10^{-12} Cu-m (S)	33	0.06 ± 0.11	31 ± 0.65

4. CONCLUSIONS

These first experiments show that potentized nanodilutions (PNDs) of metallic copper have biological effects on pea seed development which are similar to the effect of copper (water solutions of $CuS0_4$) - suppression of root and stem formation and growth. The results, also, show that PNDs can change the response of seeds to the applied stress stimulating their response to overcome stress. In medicine the therapeutic effect of potentized solutions is based on the principle of similarity, which was observed too in our experiments. Presented

results indicate that pea is promising to develop a plant model to study the effects of potentized nanodilutions and can contribute to the attempts to establish plant based bioassays.

Table 6. Development of stem on the 5th day after PND treatment

N	Variant for seed germination	Stem length [cm]	Stem weight [mg]
1	H_2O, control	2.63 ± 0.08	197± 0.90
2	10^{-2} mol $CuSO_4$	0.77 ± 0.23	36 ± 1.12
3	10^{-2} mol $CuSO_4$ + $10^{-20\,000}$ Cu-m	0.12 ± 0.25*	57.0 ± 0.74*
4	10^{-2} mol $CuSO_4$ + $10^{-2\,000}$ Cu-m on 0 h	0.13 ± 0.07*	39 ± 0.62
5	10^{-2} mol $CuSO_4$ + 10^{-400} Cu-m on 0 h	0.13 ± 0.06	32 ± 0.18
6	10^{-2} mol $CuSO_4$ + 10^{-60} Cu-m on 0 h	0.13 ± 0.08*	59 ± 0.80*
7	10^{-2} mol $CuSO_4$ + 10^{-60} Cu-m (S)	0.98 ± 0.16	40 ± 1.16*
8	10^{-2} mol $CuSO_4$ + 10^{-24} Cu-m (S)	0.70 ± 0.17	55 ± 0.95*
9	10^{-2} mol $CuSO_4$ + 10^{-12} Cu-m (S)	0.12 ± 0.0	39 ± 0.0

Legend to Tables 5 and 6: S – Serial treatment when a drop of Cu-m was added immediately (0 h) and on the 1st, 4th, 24th and 48th hour; * Statistically significant from control (10^{-2} mol $CuSO_4$) at P = 0.05.

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