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ORIGINAL PAPER

Stimulative influence of germination and growth of maize seedlings originating from aged seeds by 2,4-D potencies

Vesna Dragicevic[1,*], Mihajlo Spasic[2], Milena Simic[1], Zoran Dumanovic[1] and Bogdan Nikolic[3]

[1]*Maize Research Institute "Zemun Polje", Slobodana Bajica 1, 11185 Zemun Polje, Serbia*
[2]*Institute for Biological Research "Sinisa Stankovic", Bld. Despota Stefana 142, 11060 Belgrade, Serbia*
[3]*Institute for Plant Protection and Environment, Teodora Drajzera 9, 11040 Belgrade, Serbia*

Background: **The 2,4-D (2,4-dichlorophenoxyacetic acid) is using as a growth regulator in tissue culture media. Maize seeds have poor ability to maintain germination rate in the long term.**
Objective: **To examine the possible restorative effect of homeopathic 2,4-D potencies on maize seedlings originating from seeds damaged by accelerated aging.**
Methods: **Seeds of four maize lines were subjected to accelerated aging stress treatment. Seed samples were treated with distilled water (control) and a range of potencies of 2,4-D: 3C, 3.75C, 4.5C, 5.25C and 6C. The germination capacity, fresh substance (FS) and length of root and shoot were determined. Hydrolysis and biosynthesis, GSH/GSSG ratio and redox capacity were calculated.**
Results: **Induced seed aging decreased germination rate and growth of seedlings. 2,4-D potencies did not have a statistically significant effect on germination. However, there were statistically significant effects on FS production, root and shoot length and redox capacity. The 3C potency had the largest effect on the FS accumulation, 4.5C increased root and shoot length, compared to control (statistically significant). The GSH/GSSG ratio and the redox capacity were decreased by aging. The 3C and 4.5C potencies tended to reverse the GSH/GSSG ratio (statistically significant) in the root and shoot, (*i.e.*, shifted the redox balance to the reduced state).**
Conclusion: **Homeopathic potencies of 2,4-D appear to have a beneficial effect on artificially aged maize seeds: they stimulate growth through better substance conversion from seed rest, and shift the redox capacity towards a reduced environment. Further work is required to determine if this is an useful means of improving maize seed germination and growth.** *Homeopathy* (2013) **102**, 179–186.

Keywords: Maize; 2,4-D; Seed aging; Growth; Redox capacity

Introduction

The holistic approach defines seeds as biological systems with living processes reduced to the minimum in order to sustain germination ability (viability). In some cases, valuable material has a poor ability to maintain viability for a long periods. If environmental factors impede breeding, the application of methods which could increase vigour, accelerate germination and produce strong plants that could improve yield could be of great interest. Germination starts with water uptake and terminates with the elongation of the embryonic axis,[1] which has as a consequence protrusion of the root, and later of the shoot. Subsequent events, including the mobilization of the major seed reserves by hydrolysis processes (occurring in the seed) and biosynthesis (substance allocation to the root and shoot) are associated with growth.[2] Moreover, germination is closely tied to the redox balance, emphasizing the role of glutathione.[3,4]

*Correspondence: Vesna Dragicevic, Maize Research Institute "Zemun Polje", Slobodana Bajica 1, 11185 Zemun Polje, Serbia. E-mail: vdragicevic@mrizp.rs
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The loss of seed vitality during long-term storage is the consequence of many spontaneous and damaging processes,[5] including production of reactive oxygen species, which damage the cell parts, leading to a slowing of respiration and membrane disintegration,[6] and glutathione oxidation.[7] Schafer and Buettener, for this reason, suggested a model based on relations of the GSSG/GSH couple (reduced/oxidised glutathione) for the quantification of physiological states.[8] In some cases, glutathione oxidation could lead to necrosis[9] and loss of germination ability. The negative influence of seed aging is common in maize lines, owing to high homozygosity, a consequence of continual inbreeding. This is universal practice for the achievement of desirable characteristics in the production of maize hybrids.

Homeopathy can be aimed at improving the physiological and qualitative characteristics of plants, in addition to their resistance to biotic (insects and pathogens) and abiotic (physical and chemical damage) stress.[10] Some authors underline that the potential benefits of homeopathic preparations are that they are relatively cheap (high dilutions), simple to prepare and have few or no adverse ecological effects.[10] Hamman *et al.*[11] state that plant models offer a method of examining the efficacy of homeopathically prepared solutions. They found that barley seeds from different vigour groups reacted in different way to potentialized solutions of gibberellic acid. Homeopathic dilutions of growth regulators give a range of different results, from increasing to slowing of germination to acceleration or retardation of germination and differences in the growth of the produced seedlings.

2,4-D (2,4-dichlorophenoxyacetic acid) was the first and is still one of the frequently used herbicides, highly toxic to broadleaf plants.[12] In cell culture production, it is used as a substitute for indolacetic acid.[13,14] It presents a hazard for human and animal health, causing a significant number of deaths by poisoning.[15] The toxic action of 2,4-D is due to free radical reactions that lead to DNA damage, as well as to cell apoptosis as the result of changes in the potential of mitochondria membrane. In micro-molar concentrations, 2,4-D stimulates membrane redox activity,[16] protein and RNA synthesis,[17] lowers cytoplasmic pH.[18] Paradoxically, low concentrations of 2,4-D improve viability of *Trigonella foenum-graceum* seeds in the second generation.[19]

Objective

The objective of the study was to examine the possible restorative effect of homeopathic 2,4-D potencies on the germination and growth of seedlings, originating from seeds stressed by aging, and the possibility for its use as a treatment for poorly viable or damaged seeds. We also investigated whether a biochemical mechanism based on a shifting of the redox potential of maize seedlings was involved.

Material and methods

Seed sampling and accelerated aging treatment

Seeds of two dent and two sugary lines produced as a part of the basic seed production (under conditions of hand pollination, to avoid the penetration of pollen of other genotypes) in the experimental field of the Maize Research Institute 'Zemun Polje', ZP PL 175 (L1) and ZP PL 188 (L2) dent inbreeds, and ZP PL 51(L3) and ZP PL 67 (L4) as sugary lines, were examined. Seeds from middle part of maize ear, with similar shape and dimension (uniform seeds), without visible damage were subjected to accelerated aging treatment (AA)[20] at a temperature of 42°C at relative air humidity of 100% for 3, 6 and 9 days, down to a significant germination decrease (further treatment induced complete loss of germination ability). Significant germination drop was attained after 9 days for L1 (from the initial 92.5% to 41.0%), after 6 days for L2 (from the initial 89.0% to 15.2%), while after 3 days for L3 and L4 (L3 germination decreased from the initial 28.7% to 13.5%, while L4 germination decreased from 88.5% to 77.0%, and further aging decreased it to below 2%).

Preparation of 2,4-D solutions

1 l of mother solution (MS), containing 1 mol l^{-1} of 2,4-D (Sigma D 7299, Plant Culture Tested), was prepared the day before the germination test by its dissolution in distilled water (1 drop of ethanol was added to accelerate the dissolution process). According to Shimabukuro *et al.*[21] and Close and Ludeman,[22] 1 and 5 μmol 2,4-D solutions, respectively, have no physiological activity on maize tissue cultures. For this reason, 3C was chosen as the highest concentration to be tested.

We combined decimal and centesimal dilutions to prepare the desired potencies. The potencies were prepared freshly by diluting 1 ml of the MS in 99 ml of distilled water (working solution 1), 0.5 ml of the MS in 99.5 ml of distilled water (working solution 2). Working solution 3 was prepared by diluting 1 ml of the MS 1 in 9 ml of distilled water and working solution 4 by diluting 1 ml of the MS 2 in 9 ml of distilled water. Then a series of incremental 1/100 dilutions were prepared with vigorous mechanical shaking in Erlenmeyer flasks (500 ml; Boral, Pula, Croatia) on an in-house (Maize Research Institute) rotating shaker at 200 rpm for 5 min. The chosen working dilutions prepared from working solution 1, were 3C (10^{-6} mol l^{-1} 2,4-D in the final sample assay), and 6C (10^{-12} mol l^{-1} 2,4-D in the final sample assay). The chosen working dilution prepared from working solution 3 was 4.5C (10^{-9} mol l^{-1} 2,4-D in the final sample assay); from working solution 2 was 3.75C (5×10^{-8} mol l^{-1} 2,4-D in the final sample assay) and from working solution 4 was 5.25C (5×10^{-11} mol l^{-1} 2,4-D in the final sample assay).

Experiment I – germination test

Using a sheet of filter paper (PK 60 g m^2, 60 × 60 cm; Papírna Pernštejn spol. s.r.o., Pernštejn, Czech Republic), as the germination medium, the seeds were soaked in distilled water, as the control (without succession) to test germination under standard conditions, and in the prepared 2,4-D potencies 3C, 3.75C, 4.5C, 5.25C and 6C. Subsequently, the germination capacity was determined