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SHESTOPAL O.L. [1], ZAMBRIBORSHCH I.S. [1], IGNATOVA S.O. [1], SHPAK D.V. [2]
[1] Plant Breeding & Genetics Institute – National Center of Seed and Cultivar Investigation,
Ukraine, 65036, Odessa, Ovidiopolskaya road, 3, e-mail: izambriborsh@gmail.com
[2] Rice Research Institute at Ukrainian Academy of Agrarian Sciences,
Ukraine, 75705, Kherson region, Skadovsk district, Antonovka village, e-mail: shpak_dmitry@mail.ru

EFFECT OF PANICLE PRETREATMENT ON MORPHOGENESIS IN ANTHER CULTURE *ORYZA SATIVA* L.

Aims. Study of the influence pretreatment of panicle on morphogenic potential of microspores in anther culture of rice. ***Methods.*** Obtaining of rice double haploid lines by anther culture *in vitro*. The statistical methods. ***Results.*** The influence different variants pretreatment of rice panicle on the processes of induction and regeneration in anther culture of rice were studied. The 218 green plants-regenerants were received. ***Conclusions.*** To increase the formation of embryo like structures in anther culture of rice panicles should be incubated for the pretreatment in water at 6–8°C during 3–7 days. The positive effect on the formation of embryoides and sufficient amount of green regenerants and the low formation albino regenerants from using the cold pretreatment of panicle by solution abscisic acid (0.5 mg/L) were showed.
Key words: rice hybrids, anther culture *in vitro*, double haploid.

UDC 581.5+57.04
SAKHNO L.O.
Institute of Cell Biology and Genetic Engineering NAS of Ukraine,
Ukraine, DSP-22, 03680, Kyiv, Zabolotnogo str., 148, e-mail: sakhno@icbge.org.ua

SEED INTERFERON IMBIBITION LEADS SEEDLING GROWTH ENHANCEMENT ACCOMPANYING BY INCREASE IN SUPEROXIDE DISMUTASE ACTIVITY

Interferons (INFs) are proteins induced in the genome of vertebrates by viruses, double-stranded RNA, and some other agents [1]. It was shown unfractionated human leukocyte INF, as well as highly purified subspecies of this INF (alpha and beta), and a purified recombinant of human leukocyte INF produced in bacteria was active in suppressing multiplication of tobacco mosaic virus (TMV) in tobacco (*Nicotiana tabacum* L.) leaf discs [2]. INF-gamma application increased cytokinin activity and induced synthesis of pathogenesis–related and heat shock proteins in tobacco and wheat (*Triticum aestivum* L.) tissues [3]. Human INFs (alpha, beta, and gamma) antiviral activities were shown in datura *Datura stramonium* L. infected by TMV and globe amaranth *Gomphrena globosa* L. inoculated by potato virus X [4]. Some studies were conducted for *inf* gene expression in plants to obtain ones with improved virus tolerance [5]. Numerous researches were focused to produce recombinant INFs in plants [6]. But INF effects did not investigate at whole plant level under non-stress conditions.

In animal cells exogenous INF-alpha application is accompanied by superoxide radical formation and superoxide dismutase (SOD) activity increasing. Thus, preincubation of intact human neutrophils with INF-alpha and subsequent stimulation with calcium ionophore A23187 significantly enhanced superoxide radical generation that reduced nitroblue tetrazolium into blue formazan [7]. Mouse (L, L929, L1210 S6, and L1210 R3) and a human (WISH) cell lines pretreated with homologous INF and different

concentrations of diethyldithiocarbamate (DDC) for various periods of time were also tested for their ability to support virus multiplication. Treatment of cells with DDC resulted in dose- and time-dependent inhibition of SOD activity and, simultaneously, in the reduction of antiviral protection by exogenous INF [8]. In addition, the antioxidant activity of INF-alpha was examined on rat hepatocytes undergoing oxidative stress and hepatic stellate cells (HSCs) in primary culture as well as isolated rat liver mitochondria [9]. INF-alpha activity led to dose-dependent increase of the immunoreactive protein levels of copper, zinc- and manganese-dependent SOD both in stressed hepatocytes and activated HSCs.

In plants elevated SOD activity is related with enhanced tolerance to various stresses in term of larger plant biomass which resistant cultivars or *sod* overexpressing plants can form in comparison with sensitive or wild-type ones [10, 11]. The aim of our study was to estimate effect of recombinant human INF-alpha application on canola seed germination and seedling growth by using fresh weight, total soluble protein content, and SOD activity evaluation.

Materials and methods

Plant material and INF treatment. Seeds of spring canola (*Brassica napus* L.) cv Lega were surface sterilized, dried by filter paper and imbibed on water INF solutions with different concentrations (10^2, 10^3, 10^4 IU/ml) for 30 min. Seeds were kindly provided by Slisarchuk M.V. (Department of linseed and rapeseed selection and seed production of National Scientific center "Institute of UAAS on agriculture"). There were three control treatments namely 1) pure water, 2) 10^2 IU/ml INF inactivated by boiling, 3) 10^2 IU/ml INF with 2 mM DDC. Then seeds were dried by filter paper again and placed into Petri dish with agar solidified MS [12] medium without hormones. Ten seeds were incubated in each Petri dish. Germination and seedling growth took place in thermostat at 24 °C. Laferon (recombinant human interferon alpha 2b, 1 MIU, Inter-Pharm-Biotek, Ukraine) was used for INF solution preparation. Seedling total fresh weight (FW), FW of cotyledons, hypocotyls and roots were measured using the scale Pioneer™ PA413C (Ohaus Corporation, USA).

The total soluble protein (TSP) content was determined using Bradford method [13]. The extracts from plant leaves were prepared in triple volume of 100 mM Tris/HCl buffer, pH 8.0. The optical density was detected at 595 nm by BioPhotomether Eppendorf, v.1.35 (Germany).

SOD activity was measured using photochemical oxidation of nitro blue tetrazolium method [14]. Fresh plant material (100 mg) was pounded with 1 ml of Tris-HCl buffer (pH 8.0) in mortar and centrifuged at 13000 g (4°C) for 15 min. The supernatant was used for analyses. Formazan formation was held in Eppendorf tube (1.5 ml). Plant extracts could inhibit this reaction due to SOD activity. One tube for each probe was retained in the dark. The others were illuminated with white light lamp (fluorescent lamp T5/G5, model ELI-230A-T5-8W) during 5 min in the thermostat at 24°C. Null probe had no leaf extract in its composition. In this probe oxidation was complete. The optical density of illuminated probe was measured at 550 nm by BioPhotomether Eppendorf (Germany) versus the optical density of dark probe.

Statistical analysis was performed according to Duncan multiple range test. Differences from control values were significant at $p \leq 0.05$. Three independent experiments were conducted in five replications. There were nine replications for formazan measurement.

Results and discussion

Canola seed germination started two days after experiment beginning. No differences in germination were detected between controls and INF treated seeds (fig. 1, A). Hypocotyls and roots of experimental seedlings were longer in comparison with controls (fig. 1, B). Seedlings which obtained from 10^3 IU/ml INF treated seeds were larger ones produced from seeds treated by 10^2 IU/ml INF (fig. 1, B) and 10^4 IU/ml INF solutions.

In seven-day-old seedlings total FW, FW of cotyledons, hypocotyls and roots were measured and these parameters for single seedling were calculated (fig. 2, A). INF application stimulated seedling biomass accumulation in canola up to 2, 2.4 and 1.7-fold after the imbibition with 10^2, 10^3 and 10^4 IU/ml INF solutions, respectively, in terms of higher FW of hypocotyls and roots.

Differences in cotyledon TSP were not determined, but in hypocotyls TSP was 2 times higher in control seedlings in comparison with treated ones (fig. 2, B).

SOD activity was significantly lower in control seedlings (both cotyledons and hypocotyls) in comparison with INF treated ones (fig. 2, C). Moreover the highest SOD activity was detected in seedlings obtained from 10^3 IU/ml INF treated seeds. It was up to 2.18- and 1.47-fold higher in cotyledons and hypocotyls, respectively, in the latter in comparison with controls).

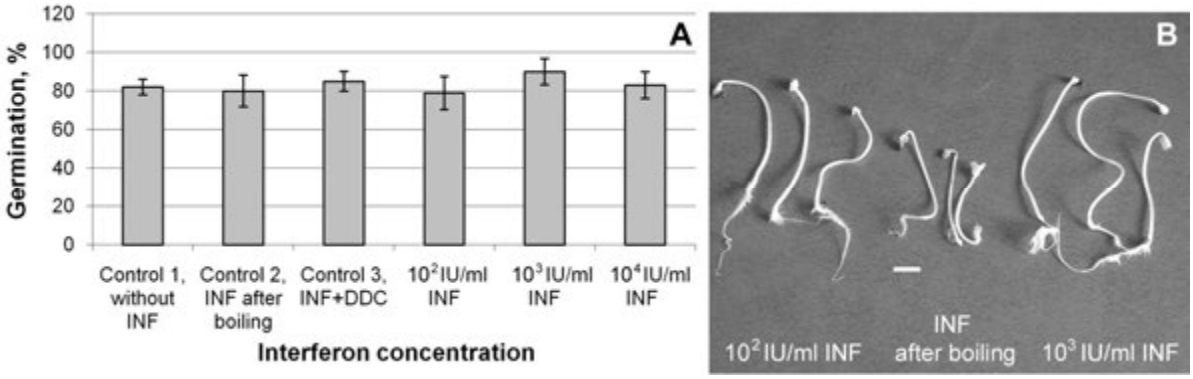

Fig. 1. Canola cv Lega seed germination (**A**); 7-day-old seedlings were grown on agar solidified hormone free MS medium in thermostat at 24 °C after seed treatment by water solutions with 10^2 IU/ml INF (left), INF inactivated by boiling (middle), and 10^3 IU/ml INF, (right) (**B**). *Scale bar*: 1 cm

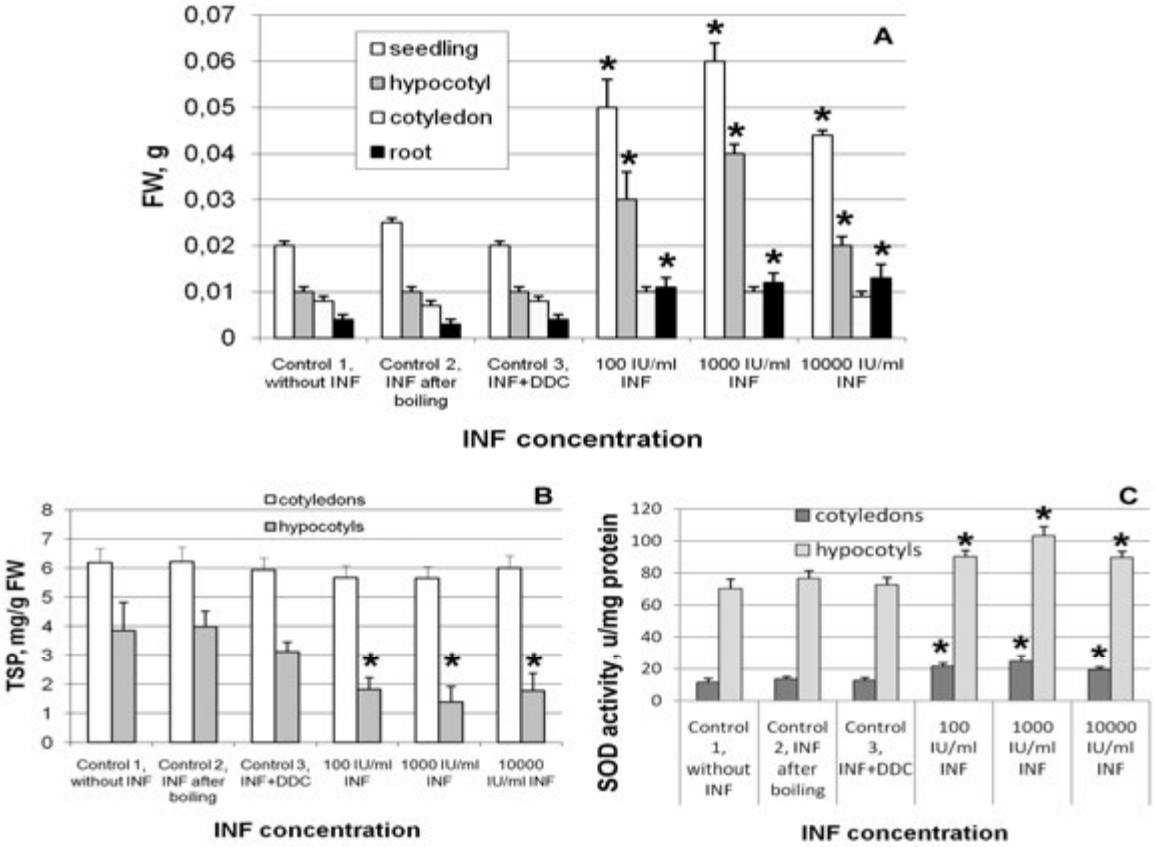

Fig. 2. Physiological and biochemical parameters of canola seedlings after INF imbibition (growth in thermostat at 24 °C): **A** – fresh weight of single 7-day-old seedling, **B** – cotyledon and hypocotyl total soluble protein content, **C** – SOD activity in cotyledons and hypocotyls. *Error bars* represent mean±one standard deviation and *asterisk* * indicates significant differences between experimental values compared with control ones ($p \leq 0.05$)

It was shown plant growth could be affected by treatments using substances both plant and animal origin. Thus, the growth of *Arabidopsis thaliana* L. seedlings was promoted by progesterone (mammalian gonadal hormone) at low concentrations but suppressed at higher concentrations under both light and dark growth conditions. The growth of the gibberellin-deficient mutant of pea (*Pisum sativum*) was also promoted by progesterone [15]. Exogenous melatonin

treatment of wild type rice as well as it accumulation due to sheep serotonin N-acetyltransferase overexpression in transgenic one enhanced root growth by seminal root elongation [16]. Under non-stress conditions SOD activity increment by 10 % was detected in transgenic rice in comparison with wild-type plants. The melatonin-treated maize (*Zea mays* L.) plants produced up to 20 % more corn than controls [17]. Salicylic acid induced the synthesis of antioxidant enzymes (SODs) in treated Arabidopsis seedlings and improved their salinity tolerance [18]. In our canola seedlings FWs of control ones were lower in comparison with INF treated. Moreover INF dose-dependent increase in SOD activity was also detected in the latter.

SOD gene overexpression led to biomass enhancement both in favourable and stress conditions in laboratory as well as under field trials in alfalfa *Medicago sativa* L. [19], canola [20], rice *Oryza sativa* L. [21], cotton *Gossypium barbadense* L. [22]. In plants expressing *huINF*б2b gene SOD activity increase under non-stress *in vitro* conditions was detected in transgenic canola [23] and chicory (*Cichorium intybus* L.) [24]. We propose exactly heterologous gene expression resulting recombinant human INF-alpha accumulation affects SOD activity and others physiological and biochemical parameters in these plants.

Thus, substances which involve changes in SOD activity could affect seedling growth. Produced in bacteria human recombinant INF-alpha 2b caused FW improvement in canola seedlings.

Conclusions

Human recombinant INF-alpha 2b application caused an dose-dependent increase in canola seedling biomass (up to 2.4-fold) during *in vitro* growth. SOD activity rise and its positive correlation to FW accumulation were detected for these seedlings. We suppose SOD activity increment which occurred due to seed INF imbibition improved plant growth.

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SAKHNO L.O.
Institute of Cell Biology and Genetic Engineering NAS of Ukraine,
Ukraine, DSP-22, 03680, Kyiv, Zabolotnogo str., 148, e-mail: sakhno@icbge.org.ua

SEED INTERFERON IMBIBITION LEADS SEEDLING GROWTH ENHANCEMENT ACCOMPANYING BY INCREASE IN SUPEROXIDE DISMUTASE ACTIVITY

Aim. We studied canola (*Brassica napus* cv Lega) *in vitro* seedling growth after seed imbibition by human recombinant interferon alpha 2b (INF) produced in bacteria. ***Methods.*** Germination, seedling fresh weight (FW), total soluble protein (TSP), and superoxide dismutase activity (SOD) measurements were conducted. ***Results.*** INF applications did not improve canola seed germination. TSP was not affected in cotyledons and decreased in hypocotyls. Seedling FW was increased by 100 % (10^2 IU/ml INF), 140 % (10^3 IU/ml INF), and 70 % (10^4 IU/ml INF) in comparison with controls (treatments by pure water, or INF after boiling, or INF with diethyldithiocarbamate) under growth in thermostat at 24°. Differences in seedling FW were due to differences in hypocotyl and root FWs. SOD activity increased up to 2.18-fold in cotyledons and 1.47-fold in hypocotyls. ***Conclusions.*** Human recombinant INF alpha 2b application caused an dose-dependent increase of canola seedling biomass (up to 2.4-fold) during *in vitro* growth. SOD activity rise and its positive correlation to FW accumulation were detected in these seedlings. We suppose SOD activity increment which occurred due to seed INF imbibition improved plant growth.
Key words: canola, fresh weight, interferon, superoxide dismutase activity.