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SHORT COMMUNICATIONS

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# Increasing of antioxidant and superoxide dismutase activity in chicory transgenic plants

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***Aim***. *Determination of the antioxidant activity (AOA) and superoxide dismutase (SOD) activity in transgenic chicory plants carrying the human interferon α2b target and nptII or bar selective genes.* ***Methods***. *AOA was measured by a method based on the determination of kinetics of the reduced 2,6-dichlorophenolindophenol oxidation. SOD activity was assayed using the system consisting of methionine, riboflavin, and nitroblue tetrazolium.* ***Results***. *Antioxidant activity of transformed plants extracts was more than 1,91–2,59 and 2,04–2,43 times over the activity of control non-transgenic plants (at nptII and bar gene presence respectively). SOD activity was higher in transgenic plants than in the control, and was 2,03 ± 0,46–3,33 ± 0,54 U/g weight (nptII gene) and 2,25 ± 0,46–2,68 ± 0,08 U/g weight (bar gene).* ***Conclusions***. *Transgenic C. intybus plants have higher antioxidant and superoxide dismutase activity compared to non-transgenic plants. The increasing of AOA and SOD activity is a response of plants to transformation stress factor and integration of foreign genes in plant genome.*

*Keywords: genetic transformation, Cichorium intybus, antioxidant activity, superoxide dismutase activity.*

**Introduction.** Genetic transformation is used to create transgenic plants for scientific purpose (for instance, investigation of gene functioning) and for obtaining plants, synthesizing new substances. However, recently the safety issues of using plants with artificially modified genome have become rather urgent [1, 2]. Physiological and biochemical properties of plants may change after the transformation [3].

There are the data, testifying to the stress of the transformation process, *Agrobacterium*-mediated, in particular, for plants at each stage [4]: *in vitro* cultivation, injury, contact with microorganism, selection, transfer of the foreign gene to the genome of plants, synthesis of corresponding proteins, biological activity of the protein. One of the reactions to the activity of stress agents is known to be the activation of antioxidant systems of plant protection, namely, the increase in the number of low-molecular antioxidants and the activity of enzymes (superoxide dismutase (SOD), catalase, peroxidase, etc.) [5, 6]. In particular, reactive oxygen species are formed and accumulated at the effect of drought, temperature stress, etc. which leads to oxidative stress. These changes are accompanied by the increase in the activity of the antioxidant system [5]. Taking the abovementioned into account it would be interesting to investigate whether there are any changes in the activity of the antioxidant system and its components, induced by the transfer of the gene (genes). The current work is aimed at determining the antioxidant activity (AOA) and the activity of SOD of transgenic *C. intybus* L. plants with human interferon-α2b (*ifn-α2b*) gene and different selective *nptII* and *bar* genes.

**Materials and Methods**. The material for the investigation was transgenic *C. intybus* var. *foliosum* Hegi plants with target *ifn-α2b* gene and selective *nptII*

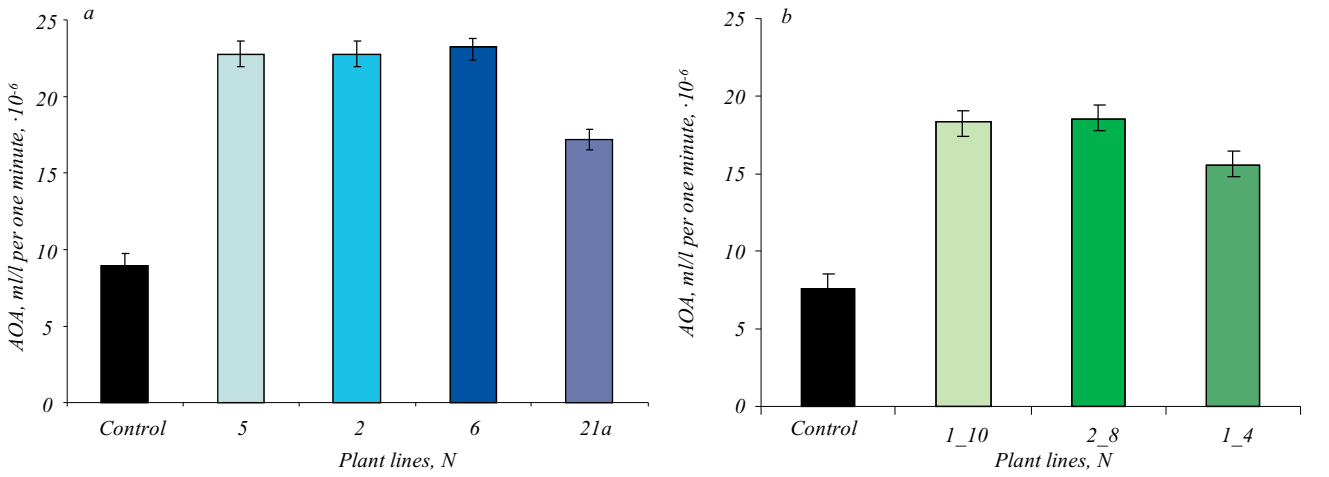


Fig. 1. The antioxidant activity of transgenic chicory plants with *ifn-α2b* and *nptII* (*a*) and *ifn-α2b-bar* (*b*) genes

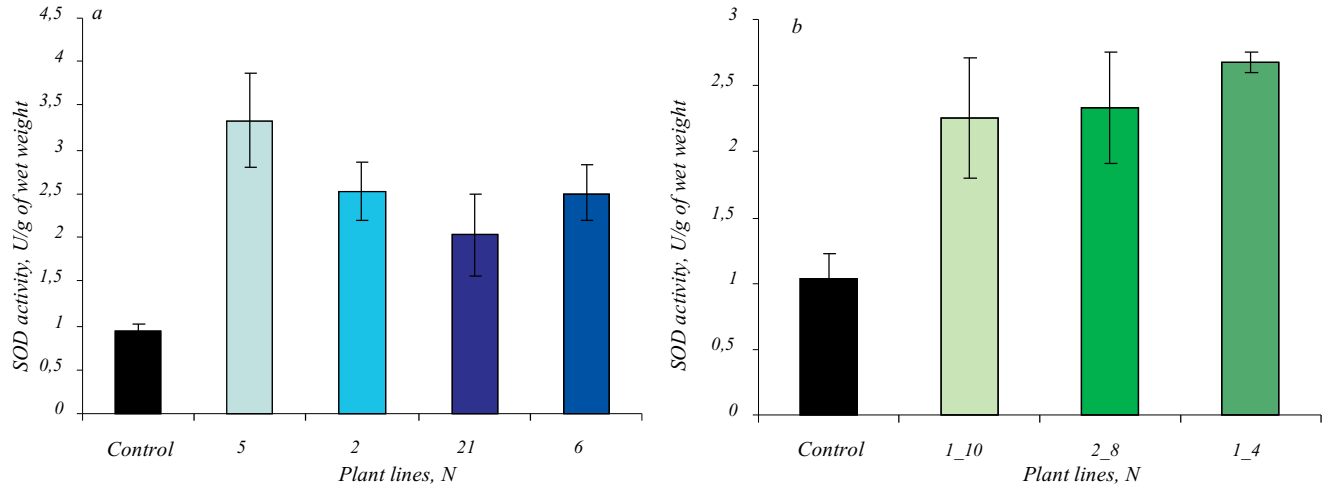


Fig. 2. The activity of superoxide dismutase in transgenic chicory plants with *ifn-α2b* and *nptII* (*a*) and *ifn-α2b-bar* (*b*) genes

(four lines) and *bar* (three lines) genes, previously obtained by us [9, 10]. Regenerant plants were cultivated in Murashige and Skoog medium [11] with twice decreased concentration of macroelements for 30 days. AOA of plants was determined by the method, described in [12] with modifications, SOD activity – as described in [13]. The experiment results were statistically processed using variance analysis of single-factor experiment.

**Results and Discussion**. AOA of the extracts of transgenic chicory plants was considerably higher than that of non-transformed plants (Fig. 1, *a*, *b*). For instance, AOA of the extract of transgenic lines with *ifn-* α*2b–nptII* genes was 17.15 ± 0.66–23.20 ± 0.60 ml/l per one minute (control – 8.97 ± 0.79), while that for extracts of plant lines with *ifn-α2b–bar* genes – 15.63±0.89–18.6 ± 0.84 ml/l per one minute (control – only 7.67 ± 0.84). Disperse *Ff* ratio (219.66 and 91.55 for plants with *ifn-α2b–nptII* and *ifn-* α*2b–bar* genes, respectively) was considerably higher than the critical point *Fst* (2.5 and 7.59, respectively, with 5 % significance point). Therefore, the differences obtained are statistically reliable at the level of reliable probability $P_{095}$.

The activity of SOD extracts of all plants with genes *ifn-α2b–nptII* turned out to be higher compared to the control, respectively, from 2.3 ± 0.6 to 3.33 ± 0.54 and 0.94 ± 0.07 U/g of fresh weight (Fig. 2, *a*). At the same time, the disperse ratio *Ff* was 0.89 which is much less than *Fst* (5.99 with 5 % significance point).

Therefore, the reliability of obtained differences in SOD activity is not confirmed. Here the comparison of factorial and residual dispersion testifies to the necessity of increasing the sample to confirm the reliability of present differences.

The activity of SOD extracts from plants with *bar* gene is 2.25 ± 0.46–2.68 ± 0.08 U/g of wet weight which is 2.17–2.58 times higher that the activity in the control (Fig. 2, b). Disperse *Ff* ratio is 12.25 which is much higher than *Fst* (7.59 at $P_{095}$). The differences in SOD activity of transgenic and control plants are statistically reliable at the mentioned level of significance. Thus, transgenic chicory plants differ from the control by the increased level of the antioxidant activity and SOD.

*In vitro* cultivation may be excluded from possible reasons of changes in AOA and SOD activity in transgenic chicory plants as both transgenic and control plants were cultivated *in vitro* and they passed the regeneration stage. The contact with bacteria and selection are likely not to condition the variation in AOA and SOD activity in transgenic plants as the investigations were conducted two years after obtaining them. The changes in AOA and SOD activity occur in plants with genes *nptII* and *bar*. The type of the selective gene is not likely to cause any changes.

The analysis of obtained plants for the presence of transgenes demonstrated that the transfer of both target and selective genes occurred for all the investigated lines. Thus, the increase in AOA and SOD activity by transformed plants may be related to the presence of foreign genes. The transcription of *ifn-α2b* gene was revealed only in one line of plants with *nptII* gene and in all the plants with *bar* gene. Therefore, there are no reasons to state that the transcription of transgenes is the reason of the increase in AOA and SOD activity in transgenic plants.

The plants with the increased AOA level and SOD activity are more resistant to oxidative damage, caused by the effect of stress factors – drought, salinization, etc. [14, 15]. Therefore, the obtained transgenic chicory plants with high AOA level may be more resistant to the effect of the mentioned stress factors and thus used in biotechnology and selection.

**Conclusions**. Transgenic *C. intybus* plants are remarkable for the increased activity of the antioxidant system and the enzyme of superoxide dismutase compared to the control non-transformed plants, which may be the response of the plant organism to the effect of transformation as a stress factor. The stress state of transgenic chicory plants is probably related to the transfer of foreign genes to the genome of plants.

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Підвищення антиоксидантної активності та активності супероксиддисмутази у трансгенних рослинах цикорію *Cichorium intybus* L.

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Summary

***Мета***. *Визначення антиоксидантної активності (АОА) та активності супероксиддисмутази (СОД) у трансгенних рослинах цикорію з геном інтерферону-α2b людини та генами nptII і bar.* ***Методи***. *АОА вимірювали методом, заснованим на визначенні кінетики окиснення відновленої форми 2,6-дихлорфенолін-дофеноляту натрію, активність СОД – за інтенсивністю інгібування тетразолю блакитного екстрактом рослин.* ***Результати***. *АОА екстрактів трансформованих рослин перевищує активність контрольних (нетрансгенних) у 1,91–2,59 і 2,04–2,43 разу (гени nptII і bar відповідно). Активність СОД виявилася вищою у трансгенних рослин і становить 2,03 ± 0,46–3,33 ± 0,54 (ген nptII) і 2,25 ± 0,46–2,68 ± 0,08 (ген bar) ум. од/г сирої маси.* ***Висновки***. *У трансгенних рослин C. intybus спостерігається підвищена активність антиоксидантної системи і СОД, що, вірогідно, є реакцією рослин на дію трансформації як стресового фактора. Стресовий стан трансгенних рослин цикорію може бути пов'язаний із перенесенням чужорідних генів до геному рослин.*

*Ключові слова: генетична трансформація, Cichorium intybus, антиоксидантна активність, активність супероксиддисмутази.*

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Повышение антиоксидантной активности и активности супероксиддисмутазы у трансгенных растений цикория *Cichorium intybus* L.

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Summary

***Цель***. *Определение антиоксидантной активности (АОА) и активности супероксиддисмутазы (СОД) в трансгенных растениях цикория с геном интерферона α2b человека и генами nptII и bar.* ***Методы***. *АОА измеряли методом, основанным на определении кинетики окисления восстановленной формы 2,6-дихлорфенолиндофенолята натрия; активность СОД – по интенсивности ингибирования тетразолия голубого экстрактом растений.* ***Результаты***. *АОА экстрактов трансформированных растений превышала активность контрольных (нетрансгенных) в 1,91–2,59 и 2,04–2,43 раза (гены nptII и bar соответственно). Активность СОД была выше у трансгенных растений и составляла 2,03 ± 0,46–3,33 ± ± 0,54 (ген nptII) и 2,25 ± 0,46–2,68 ± 0,08 (ген bar) усл. ед/г сырой массы.* ***Выводы***. *Трансгенные растения C. intybus имеют повы-*

*шенную активность антиоксидантной системы и СОД, что, вероятно, является реакцией растений на трансформацию как стрессовый фактор. Стрессовое состояние трансгенных растений цикория, очевидно, связано с перенесением чужеродных генов в геном растений.*

*Ключевые слова: генетическая трансформация, Cichorium intybus, антиоксидантная активность, активность супероксиддисмутазы.*

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