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Towards an efficient sunflower regeneration system: Evaluation of different methods for efficient *in vitro* rooting of four sunflower genotypes

Kimon Ionas[1] · **Emilija Bulić**[1] · **Aleksandra Radanović**[1] · **Ankica Kondić-Špika**[1] · **Katarina Cankar**[2] · **Dragana Miladinović**[1]*

[1]Institute of Field and Vegetable Crops, Novi Sad, Serbia
[2]Wageningen University & Research, Netherlands

*Corresponding author: dragana.miladinovic@ifvcns.ns.ac.rs

Summary: Sunflower in one of the most important oil-crops worldwide. Due to the effects of climate change, it is becoming increasingly important to discover effective and reliable ways to assist it in combating abiotic stresses. A very precise, cost-efficient and reliable way to achieve sunflower abiotic stress resilience, and specifically drought resilience, is through genetic modification. Due to sunflower's recalcitrant nature to transformation and regeneration and strong genotype dependency, it is important to establish a high efficiency regeneration protocol applicable to a wide range of genotypes. The aim of this study was to evaluate the root regeneration capacity of 4 different sunflower genotypes when cultured on 5 different rooting media with the purpose of identifying a combination of sunflower genotype and medium with the highest root regeneration rate. The percentage of rooting of different genotypes showed inter-assay variability (from 0% regeneration to 100% regeneration), however, most consistent root formation was repeatedly observed for genotypes PH-BC2-91 and HA-26-PR. Statistically significant higher root regeneration rates were observed when explants of different genotypes were cultured in MS DV medium compared to four other media used (from around 90% for MS DV to around 50% for the other media). These results further verify the intricate relationship between genotype and medium interactions. The study provides a reliable basis for establishing an efficient sunflower root regeneration protocol and paves the way for the application of biotechnological approaches in breeding and enhancing resilience to abiotic stresses.
Keywords: drought stress, IBA, indole-3-butyric acid, *in vitro*, rooting medium, root regeneration, sunflower

Introduction

Sunflower (*Helianthus annuus* L.) is one of the main oilseed crops cultivated worldwide at 30.14 million hectares and producing 58.57 million tons (FAO, 2023). Although it combats drought with its drought escape behaviour, it is highly sensitive to drought and heat stresses, especially in a climate change scenario and/or with the onset of early droughts (Debaeke et al., 2017). Therefore, due to the

rising problem of climate change, the discovery of modern approaches for climate resilience has become vital. According to related studies, drought can be managed by modifying different plant traits or by incorporating new ones that will help the plants cope with drought stress successfully (Yordanov et al., 2000; Farooq et al., 2009; Cvejić et al., 2022). Genetic modification is usually one of the highest success rate strategies and one of the most cost-efficient strategies to combat drought stress (Joshi et al., 2020; Şimşek, Isak, et al., 2024). However, sunflower is not only highly recalcitrant in terms of transformation and regeneration, but also highly dependent on genotype and media composition (Miladinović et al., 2019). Thus, identifying different sunflower lines with high regeneration and rooting ability is vital to speed up the breeding efforts. One of the first steps needed, is achieving sunflower regeneration and rooting *in vitro* efficiently in order to, further down the line, be able to genetically transform the sunflower and incorporate the desirable traits that will increase its resistance to biotic and abiotic stresses and consequently to drought. Some of the modern methods of sunflower regeneration that have been reported include organogenesis and somatic embryogenesis (Liu et al., 2014; Dagustu, 2018; Islam et al., 2021), protoplast isolation (Vasić et al., 2001; Kativat et al., 2022) and anther culture (Miladinović et al., 2016; Dagustu, 2018). Nevertheless, the existing techniques need substantial improvement because the regeneration response, both for shoot and root regeneration, is strongly affected by genotype and culture conditions (Miladinović et al., 2019). Many sunflower rooting protocols have been reported testing a variety of media and hormones (Vasić et al., 2001; Taski-Ajdukovic et al., 2006; Wang et al., 2011; Inoka & Dahanayake, 2015) but due to the complicated relationship between genotype and culture media it is vital to establish a universal protocol for root regeneration in sunflower.

The aim of this present study was to identify and evaluate the regeneration of roots of 4 different cultivated sunflower genotypes *via* organogenesis from apical shoots and nodal segments when cultured on different types of rooting media and to establish optimal combination of genotype and rooting treatment with high reliability and root regeneration rates.

Materials and methods

Shoot production

Four inbred lines, HA-26-PR, PH-BC2-91, NO-SU-12 and HA-98, obtained from the Institute of Field and Vegetable crops, Novi Sad (Serbia), were used in the study. Approximately 100 seed of each line were sterilized according to the protocol of Taški-Ajduković and Vasić (2005). After sterilization, seeds were inoculated into Erlenmeyer flasks with 100 ml of solid MS medium (Murashige & Skoog, 1962). For each genotype 12 flasks with 4 seeds were cultured for 14 days at 25 °C and 16:8 photoperiod.

Rooting treatments

After 14 days, the apical shoots and nodal segments of the plants were cut, and treated as follows:

- Control Treatment (MS K): 2 nodal segments and 2 apical shoots were cultivated in each Erlenmeyer flask with 100 ml of MS K substrate (Murashige & Skoog, 1962).
- Treatment 1 (MS DV): Shoots and segments were immersed in 1 mg/L indole-3-butyric acid (IBA) solution for 5 min (no more than ¼ of the shoot/segment was immersed in the solution). After 5 minutes, the excess solution was removed from the shoots and segments using sterile filter paper, and then 2 apical shoots and 2 nodal segments were inoculated in an Erlenmeyer flask with 100 ml of MS medium (1/2 Macro nutrients, 6 g/l agar, 10 g/l sucrose) with 2,0 mM Ammonium ferric citrate, 2,0 mg/l $AgNO_3$, 50,0 mg/l Phlorizin and 3,0 g/l Casein hydrolysate (Vasić et al., 2001) (Figure 2).

– Treatment 2 (MS IBA): Shoots and segments were immersed in 0.1 mg/L IBA solution for 4 min (no more than ¼ of the shoot/segment was immersed in the solution). After 4 minutes, the excess solution was removed from the shoots and segments using sterile filter paper, and then 2 apical shoots and 2 nodal segments were cultivated in each Erlenmeyer flask with 100 ml of standard MS medium (20 g/l sucrose) (Taski-Ajdukovic et al., 2006).
– Treatment 3 (MS W): 2 nodal segments and 2 apical shoots were inoculated in an Erlenmeyer flask with 100 ml of MS medium (1/2 Macro and Micro nutrients, 10 g/l sucrose) with 0,6 mg/l of IBA (Wang et al., 2011).
– Treatment 4 (MS ID): 2 nodal segments and 2 apical shoots were inoculated in an Erlenmeyer flask with 100 ml of MS medium (20 g/l sucrose) with 1,0 mg/l of IBA (Inoka & Dahanayake, 2015).

For each treatment, 4 Erlenmeyer flasks were used with 4 explants, 2 nodal segments and 2 apical shoots. In total, each treatment consisted of 16 explants.

Experiment setup

The experimental setup was structured as follows: Three (3) initial trials (Trials 1, 2 and 3) using all 4 genotypes and two (2) subsequent trials (Trials 4 and 5) using only 2 genotypes.

After the 14-day culture period, the explants of each treatment were cultured for 21 days in growth chamber at 25°C, 16:8 photoperiod and the percentage of root formation was observed after 7, 14 and 21 days of culture (Trial 1). For Trial 2, nodal segments and apical shoots were cut from the developed explants of Trial 1 and were treated and cultured again in the same way that was described previously. The same process was repeated once more for Trial 3.

After the end of Trial 3, the experiment was repeated from the beginning using only the seeds of the genotypes PH-BC2-91 and HA-26-PR for 2 more subsequent trials (Trial 4 and Trial 5) that were conducted in same way as the 3 initial trials.

The reasoning behind the multiple trials was to test the repeatability of the experiment and the rooting stability of each genotype after a series of trials.

After the end of each culture period of 21 days, the root regeneration of the explants was monitored in order to determine the percentage of explants with formed roots. The moment of formation of the first pair of leaves was also monitored as well as the general condition of the plants after the 21 days. The results were pooled in tables where for each explant it was recorded "1" if there was a formed root and "0" if there was not a formed root.

Statistical analysis

The obtained results were statistically analysed in order to determine:

1) how each genotype affected the rooting efficiency regardless of the substrate,
2) how each substrate, when compared to the control substrate MS K, affected the rooting efficiency of the explants
3) how the interaction between substrate and genotype influenced the rooting efficiency.

Due to the binary nature of the data, the Binomial Logistic Regression test was done to evaluate the interactions mentioned above and non-parametric Kruskal-Wallis tests (One-way ANOVA) to observe the effect of each parameter on rooting efficiency. All statistical analyses were performed on 16 valid explants per treatment. Data from explants that exhibited signs of microbial or fungal contamination during the culture periods were excluded. Also, data from explants that could not provide viable nodal segments and/or apical shoots for the next trial were excluded from the analysis. Statistical analyses were performed using the Jamovi Software (v2.6.23).

Results and Discussion

Statistical analysis

The results of Binomial Logistic Regression for each of the 5 trials are shown in Table 1. The p-values suggested that in trials 1, 2, 4 and 5 no statistically significant differences were found between each combination of genotype and substrate within these trials. In contrast, in trial 3 the observed differences between coefficients were statistically significant on a level of significance p=0.05. As seen in the Binomial Logistic Regression tests (Table 1), there are observable differences in the rooting efficiency of each substrate, genotype and combination but they do not seem to be statistically significant. Therefore, non-parametric Kruskal-Wallis tests (One-Way ANOVA) were performed for each genotype and each medium in all 5 experiments.

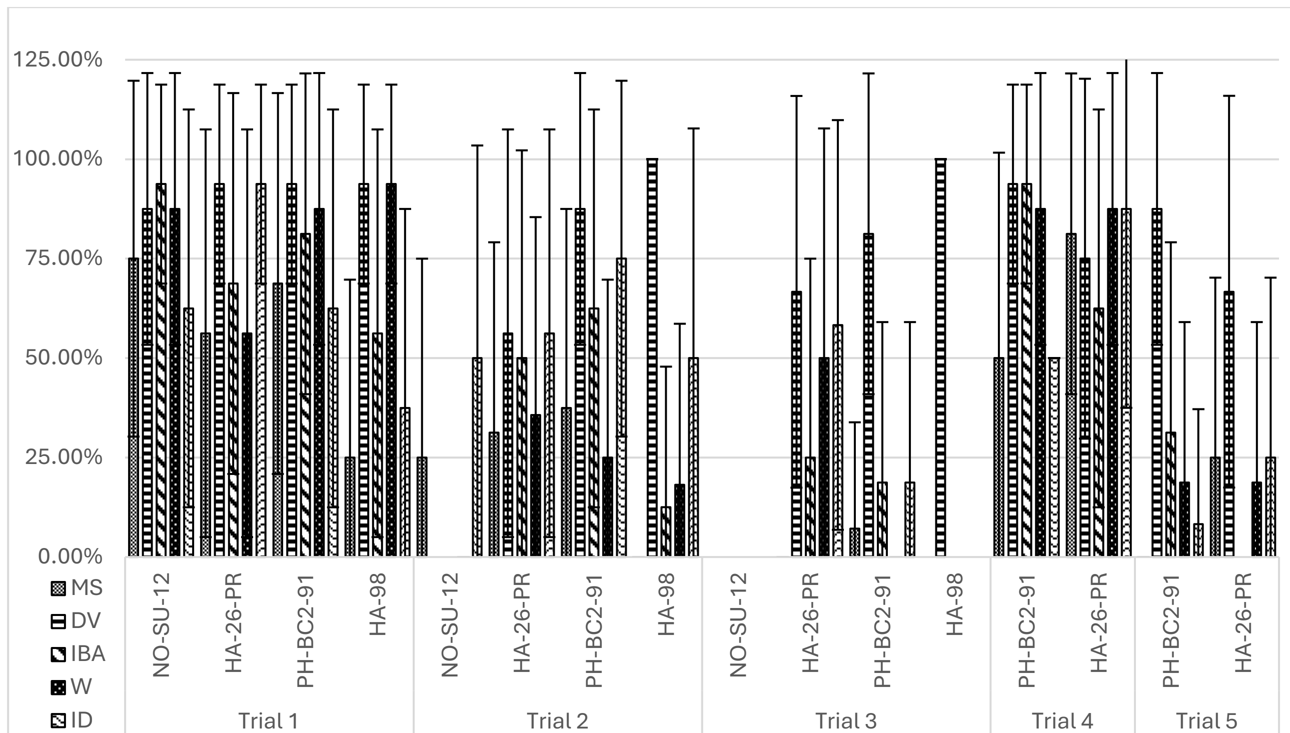

Figure 1. Root regeneration percentages with standard deviation bars of each genotype in each specific treatment among all 5 trials. NO-SU-12, HA-26-PR, PH-BC2-91, and HA-98 represent the 4 different genotypes tested. MS, DV, IBA, W and ID represent the treatments MS K, MS DV, MS IBA, MS W and MS ID respectively.

Genotypes

Genotypes HA-26-PR and PH-BC2-91 in trials 1, 2 and 4 managed to have 16/16 regenerated explants per treatment. In trial 3, HA-26-PR, besides from treatments MS DV and MS ID, had less than half of the explants per treatment (8/16 for MS K, and 4/16 for MS IBA and MS W). This was not the case for PH-BC2-91 where, besides for treatment MS W (4/16 explants), all other treatments consisted of 16/16 regenerated explants. The same situation was observed in trial 5 with PH-BC2-91 having 16/16 explants in all treatments and HA-26-PR having 12/16 in MS K, MS DV and MS ID, 16/16 in MS W and no explants in treatment MS IBA. Genotypes NO-SU-12 and HA-98 followed the same pattern in trial 1. In trial 2, NO-SU-12 had 4/16 explants in MS K and MS W and 8/16 in MS ID. HA-98 had 6/16 in MS K and MS DV, 4/16 in MS IBA and 8/16 in MS W and MS ID.

During trial 3, NO-SU-12 and HA-98 had no explants for evaluation except HA-98 in treatment MS DV (12/16). Overall, in trial 1 the best treatments for each genotype (Figure 1, Table 12) were MS IBA for NO-SU-12 (93,8%), and MS DV for PH-BC2-91, HA-26-PR and HA-98 (all at 93,8%). Similarly in trial 2, MS ID for NO-SU-12 (50%), and MS DV for PH-BC2-91, HA-26-PR and HA-98 (87,5%, 56,3% and 100% respectively). In trial 3 the best treatment was MS DV for PH-BC2-91, HA-26-PR and HA-98 (81,3%, 66,7% and 100% respectively). For PH-BC2-91 the results were the same for the subsequent trials with MS DV being the most efficient at 93,8% and 87,5% respectively for trials 4 and 5. However, HA-26PR, in trial 4 performed the best in treatments MS W and MS ID at 87,5% root regeneration but in trial 5 it was again MS DV at 66,7%.

Statistical analysis showed that in trial 1 (Tables 3a and b), there were no differences among the genotypes for each treatment besides treatment MS ID where HA-26-PR performed better than HA-98. In trial 2 (Tables 5a and b) HA-98 had better performance than HA-26-PR in treatment MS DV, but there were not other significant differences observed. In trial 3 (Tables 7a and b) there were no significant differences among the best performing treatments for each genotype. During trial 4 (Tables 9a and b) HA-26-PR performed better in treatment MS ID than PH-BC2-91 and PH-BC2-91 had higher rooting efficiency than HA-26-PR in treatment MS IBA. Lastly, there were significant differences observed in trial 4 between HA-26-PR and PH-BC2-91 (Tables 11a and b).

Apart from this experiment, genotypes PH-BC2-91 and HA-26-PR have been previously used for various studies that included *in vitro* regeneration (Vasić et al., 2000; Malenčić et al., 2004; Taški-Ajduković & Vasić, 2005; Taski-Ajdukovic et al., 2006; Taski-Ajdukovic et al., 2008; Taski-Ajdukovic et al., 2009). Similarly to this study, according to Taski-Ajdukovic et al., 2008, well developed shoots from PH-BC2-91 hybrids, successfully developed roots after being treated with IBA prior to being immersed in the culture medium. Even though the rest of the mentioned publications do not deal specifically with rooting, in all cases both genotypes showed high regeneration rates. They also demonstrated high repeatability that made them efficient candidates for experiments dealing with *in vitro* regeneration of any kind like protoplast isolation and culture (Vasić et al., 2000; Taški-Ajduković & Vasić, 2005; Taski-Ajdukovic et al., 2006; Taski-Ajdukovic et al., 2008; Taski-Ajdukovic et al., 2009). There might be an indication that due to the fact the genotypes HA-26-PR and PH-BC2-91 had regenerated explants more consistently in all treatments in the main trials, they are the most efficient overall for root regeneration. Nonetheless, the results do not clearly indicate which of the genotypes performed the best amongst all the treatments, but they do clearly show that there is a different relationship between genotype and rooting treatment emphasizing that the rooting treatment is equally as important as the genotype in root regeneration. Therefore, it is important to compare the efficiency of each genotype when the rooting treatment remains stable in order to identify specific combinations of genotype and rooting media.

Rooting treatments

Regarding what was mentioned before, it is important to observe how the different genotypes performed in comparison to each other when the treatment remained stable. The statistical analyses showed that in trial 1 (Table 2a and b), MS DV and MS W were more efficient for genotype HA-98 compared to the control treatment MS K whereas there were significant differences observed between MS K and the other treatments for the other 3 genotypes. In trial 2 (Tables 4a and b), PH-BC2-91 and HA-98 both performed better in treatment MS DV compared to the control treatment but no differences were observed for NO-SU-12 and HA-26-PR. Similarly to trial 2, in trial 3 (Tables 6a and b), significant differences were observed again between MS DV and MS K where HA-26-PR and PH-BC2-91 had higher regeneration in MS DV. Lastly, for PH-BC2-91 MS DV was also better than MS

K in trials 4 and 5 (Tables 8a,b and 10a,b), but for HA-26-PR significant differences observed in those 2 trials between MS K and the rest of the treatments.

Overall, treatment MS DV had the highest regeneration rates in all genotypes on average at 90,6% ± 29,6% in the main trials, and 81,3% ± 39,7% in the subsequent trials. The only exception was that for HA-26-PR in trial 4 MS W and MS ID were more efficient for rooting than MS DV. It was also the most consistent regarding explants regeneration where all genotypes (besides NO-SU-12) consistently had viable regenerants that could be used for the next trial. In the rest of the treatments, in the main trials, each genotype had less than half the number of explants used for each treatment.

In multiple cases, the statistical analyses showed that the tested genotypes had better root regeneration in treatment MS DV in comparison to the control treatment proving that the media composition that was used in this treatment was more efficient for root formation.

In general, the application of the published protocols that were used in this study (Vasić et al., 2001; Taski-Ajdukovic et al., 2006; Wang et al., 2011; Inoka & Dahanayake, 2015) gave similar results to those studies. Explants from all genotypes rooted well in all treatments and rooted better than in the control treatment. Compared to the control treatment MS K, the effect of IBA, both as part of the medium (MS W, MS ID) and not as part of the medium (MS DV, MS IBA), positively influenced the rooting ability of all the explants (Wang et al., 2011; Inoka & Dahanayake, 2015). Treatment MS DV positively stood out meaning that it was probably not just the usage of IBA that had an impact in the rooting ability of the explants. Vasic et al., 2001 showed that the use of silver nitrate ($AgNO_3$), phlorizin and casein hydrolysate (CH) did not only prevent vitrification of the explants but also had a positive effect on rooting similarly to this study. According to (Mahendran et al., 2019), adding silver nitrate to the medium generally improved the regeneration of plants grown *in vitro.* (Bora et al., 2018) also recorded that in *Capsicum chinense* plantlets it promoted maximum root induction in mediums containing IBA, $AgNO_3$ and GA_3. Similarly, (Siwach et al., 2012) recorded that addition of CH to MS medium (having 2.0 mg/L IBA and 0.1 mg/L IAA), significantly enhanced the *in vitro* rooting in Kinnow mandarin (*Citrus reticulata* Blanco). Additionally, (Ascencio-Cabral et al., 2008) showed that in *Carica papaya* var. Maradol when it came to root length, longer roots were produced when cultured on medium containing phlorizin, white and wide spectrum lights, especially when they were combined with phytagel and agar–agar, respectively. The results of these studies align with the results obtained from treatment MS DV and reinforce the positive effects of $AgNO_3$ and CH when combined with IBA as well as the positive effect of phlorizin when added in the medium.

Best performing genotypes on MS DV were PH-BC2-91 – 88,8% ± 31,7% and HA-98 – 97,9% ± 8,3% but there were no significant differences observed between those 2 genotypes in any trial. Genotypes NO-SU-12 and HA-26-PR also maintained a high percentage of regeneration in treatment MS DV with 87,5% ± 33,2% and 71,7% ± 44,0% respectively. Nevertheless, the results showed clear biological differences regarding regeneration efficiency. Genotypes PH-BC2-91 and HA-26-PR, consistently had a higher number of regenerated explants than HA-98 in this treatment in every trial. This could be an indicator that even though HA-98 had a slightly higher root regeneration rate genotypes PH-BC2-91 and HA-26-PR performed better biologically in treatment MS DV. Therefore, the combinations PH-BC2-91 – MS DV (Figure 3) and PH-BC2-91 – MS DV could be presented as the most efficient for rooting.

Table 1. Binomial Logistic Regression statistical analyses of all 5 trials

Model Coefficients - Root development				
Predictor	Estimate	SE	Z	p
Intercept	2.0856	0.7971	2.616	0.009*
Genotype 1	-0.4307	0.2741	-1.572	0.116
Substrate 1	-0.1019	0.2380	-0.428	0.669
Genotype 1 ✻ Substrate 1	0.0474	0.0825	0.575	0.565
Intercept	-1.1136	1.080	-1.031	0.303
Genotype 2	0.3373	0.390	0.866	0.387
Substrate 2	0.2501	0.301	0.831	0.406
Genotype 2 ✻ Substrate 2	-0.0793	0.112	-0.711	0.477
Intercept	-7.699	2.283	-3.37	<.001*
Genotype 3	2.775	0.812	3.42	<.001*
Substrate 3	2.320	0.735	3.16	0.002*
Genotype 3 ✻ Substrate 3	-0.900	0.276	-3.26	0.001*
Intercept	0.245	2.263	0.108	0.914
Genotype 4	0.318	0.881	0.361	0.718
Substrate 4	0.513	0.696	0.736	0.462
Genotype 4 ✻ Substrate 4	-0.182	0.269	-0.676	0.499
Intercept	-0.03781	2.247	-0.01683	0.987
Genotype 5	0.00662	0.867	0.00763	0.994
Substrate 5	-0.15586	0.700	-0.22268	0.824
Genotype 5 ✻ Substrate 5	-0.03903	0.274	-0.14266	0.887

The coefficient "Genotype" shows the log odds ratio associated with a change of root development per Genotype when holding the Substrate constant. A positive coefficient suggests an increased likelihood of root development for the Genotype compared to the reference level and a negative coefficient suggests a decreased Likelihood. The "Substrate" coefficient shows the log odds ratio associated with a change in Substrate while holding the Genotype constant. The positive and negative coefficients show in a similar way to previously an increase and decrease in the likelihood of root development, respectively. The coefficient of their interaction "Genotype * Substrate" represents the interaction effect between them on root development. A positive coefficient would mean that the effect of a Substrate on root development may differ depending on the Genotype and a negative coefficient would suggest the opposite. The numbers next to the coefficients indicate the number of the trial, e.g. "Genotype 1" refers Trial 1 and "Genotype * Substrate 3" refers to Trial 3. Estimates represent the log odds of " Root development = 1" vs. " Root development = 0". The p-value indicates whether there is a statistically significant difference between the parameters compared to a level of significance p=0.05.

As expected, both genotype and culture media are important for the rooting ability of sunflower explants in tissue culture. This goes in accordance with similar experiments for different species (Khanna & Raina, 1998; Zaidi et al., 2006; Şimşek, Dalda Şekerci, et al., 2024; Amankwaah et al., 2025) that prove that it is not only about the type of media or the type of growth regulators that are used but also the genetic background of the genotype and/or line that is being cultured *in vitro*.

Limitations and future prospects

This experiment was focused primarily on the root regeneration ability of genotypes and rooting media/treatments. After this is established, it would be interesting to explore further how different root qualitative traits would correlate with the results of this study.

It is worth mentioning that after the end of Trial 2, fully formed plants for Trial 3 were mostly from apical shoots. These segments were then cut into two parts (VI1 and VI2). There were no nodal segments to be cultured for trial 3 since the explants of all genotypes did not form big enough roots or roots at all making it vital to further explore or how the type of explants used affects the rooting ability of different genotypes. Furthermore, in the case of NO-SU-12, due to a combination of fungal and bacterial infections, there was not enough data for proper evaluation during trials 2 and 3. Figuring out whether this problem was genotype specific or related to the experimental procedure would be one of the next possible steps. Lastly, it would be interesting to further investigate how the quantity and the different applications of IBA actually have an effect on the rooting capacity of the explants as well as its effect on different types of explants.

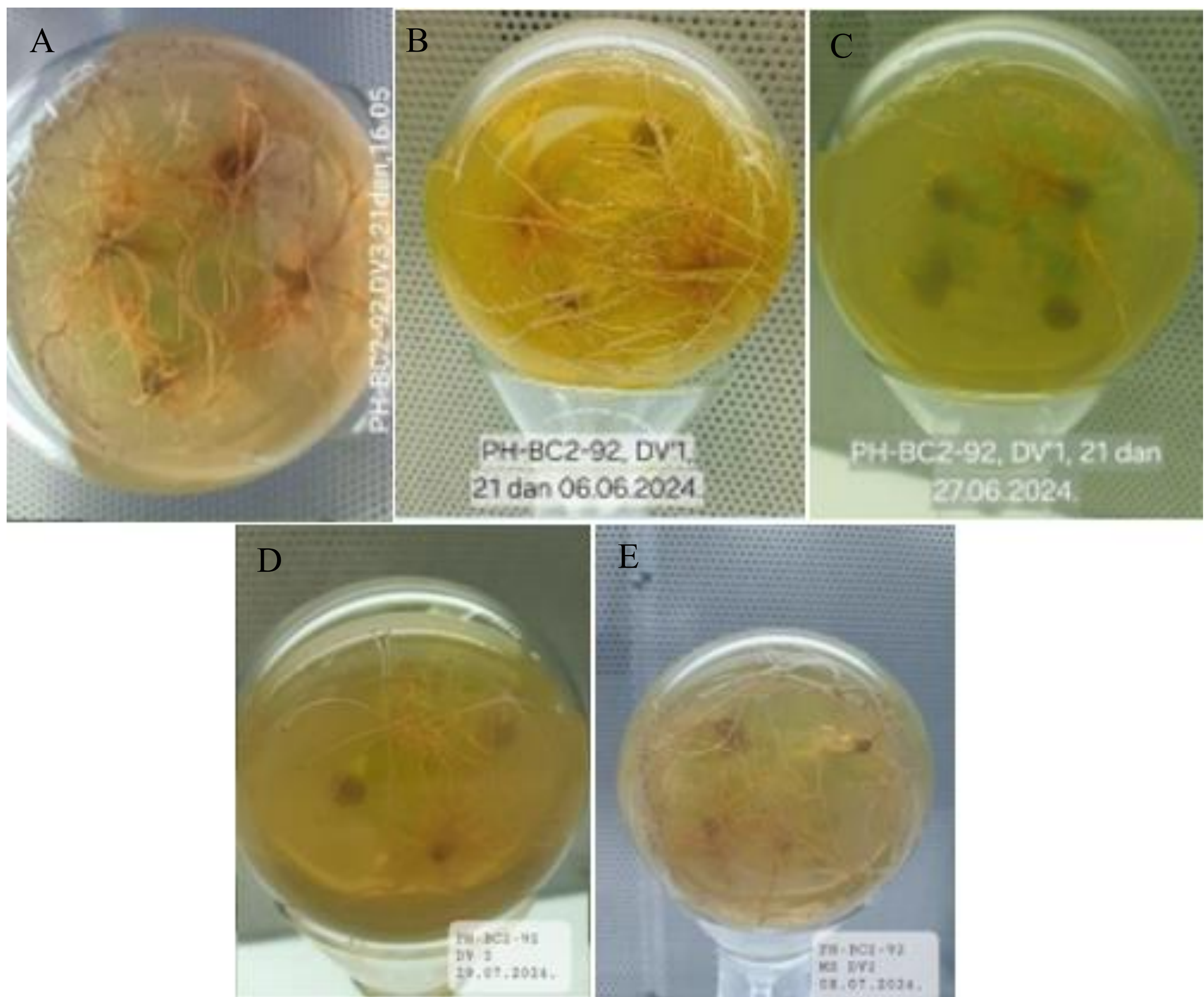

Figure 2. Roots of genotype PH-BC2-91's plants in the MS DV treatment throughout the 5 trials. A. Trial 1, B. Trial 2, C. Trial 3, D. Trial 4 and E. Trial 5. Pictures were taken 21 after the explants were immersed in the medium.

Figure 3. Representation of plants of genotype PH-BC2-91 in treatment MS DV after the end of trial 5. Pictures were taken 21 days after the explants were immersed in the medium.

Conclusion

Out of the 5 trials that were performed, treatment MS DV, when compared to the control treatment MS K, consistently showed higher percentages of root regeneration amongst all explants of all the different genotypes especially for genotype PH-BC2-91. Additionally, being the only treatment with additional components (silver nitrate, casein hydrolysate and phlorizin) it is suggested that there is a clear positive influence of these components to the rooting capability of the explants when added to the rooting medium so further exploring this relationship would be of great interest. Regarding the effect of IBA, it would be interesting to further investigate how the quantity and the way it is used, actually affect the rooting capability of the explants. On the other hand, even though there is not strong enough statistical evidence to suggest which genotype was the best for the rooting amongst all of the treatments there are biological indications that point to PH-BC2-91 and HA-26-PR having the highest rooting capability when combined with treatment MS DV. The strong relationship between genotype and culture media proves to be yet another challenge that needs to be overcome in order to achieve an efficient protocol for sunflower regeneration and transformation that can be applied to a variety of different sunflower genotypes.

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Ethical issues statement: No AI tools were used.

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Additional Data

One-Way ANOVA (Non-parametric)

Kruskal-Wallis	χ^2	df	p
HA-98	26.7	4	<.001
NO-SU-12	6.48	4	0.166
PH-BC2-91	6.34	4	0.175
HA-26-PR	11.7	4	0.019

Table 2a.4 Non-parametric test of the 4 genotypes of Trial 1. Each p-value indicates the statistical signficance of the detected differences within the genotype. Level of significance p=0.05.

Dwass-Steel-Critchlow-Fligner pairwise comparisons

	p1	p2	p3	p4
MS K - **MS DV**	<.001	0.900	0.384	0.112
MS K - MS IBA	0.391	0.604	0.930	0.952
MS K - MS W	<.001	0.900	0.714	1.000
MS K - MS ID	0.944	0.944	0.996	0.112

Table 2b. DSCF comparisons of treatments compared to control treatment MS K for each genotype of Trial 1. p1 refers to HA-98, p2 to NO-SU-12, p3 to PH-BC2-91 and p4 to HA-26-PR. The p-values indcate the stistically significant differences between each pairwise comparison. Level of significance p=0.05.

One-Way ANOVA (Non-parametric)

Kruskal-Wallis	χ^2	df	p
MS DV	0.641	3	0.887
MS IBA	6.56	3	0.087
MS W	8.88	3	0.031
MS ID	10.9	3	0.012

Table 3a. Non-parametric test for the 4 different treatments of Trial 1. Each p-value indicates the statistical significance of the detected differences within the treatment. Level of significance p=0.05.

Dwass-Steel-Critchlow-Fligner pairwise comparisons

	p1	p2	p3	p4
NO-SU-12 - HA-26-PR	0.933	0.281	0.214	0.152
NO-SU-12 - PH-BC2-91	0.933	0.719	1.000	1.000
NO-SU-12 - HA-98	0.933	0.075	0.933	0.504
HA-26-PR - PH-BC2-91	1.000	0.853	0.214	0.152
HA-26-PR - HA-98	1.000	0.890	0.075	0.005
PH-BC2-91 - HA-98	1.000	0.437	0.933	0.504

Table 3b. DSCF comparisons of genotype pairs for each treament of Trial 1. p1 refers to treatment MS DV, p2 to MS IBA, p3 to MS W and p4 to MS ID. The p-values indicate the statistically significant differences between each pairwise comparison. Level of significance p=0.05.

One-Way ANOVA (Non-parametric)

Kruskal-Wallis	χ^2	df	p
HA-98	23.5	4	<.001
NO-SU-12	-	-	-
PH-BC2-91	17.4	4	0.002
HA-26-PR	3.38	4	0.496

Table 4a. Non-parametric test of the 4 genotypes of Trial 2. Each p-value indicates the statistical signficance of the detected differences within the

Dwass-Steel-Critchlow-Fligner pairwise comparisons

	p1	p2	p3	p4
MS K - **MS DV**	0.001	-	0.033	0.626
MS K - MS IBA	0.909	-	0.633	0.861
MS K - MS W	0.818	-	0.944	0.999
MS K - MS ID	0.352	-	0.218	0.626

Table 4b. DSCF comparisons of treatments compared to control treatment MS K for each genotype of Trial 2. p1 refers to HA-98, p2 to NO-SU-12, p3 to PH-BC2-91 and p4 to HA-26-PR. The p-values indcate the stistically

genotype. Level of significance p=0.05.

significant differences between each pairwise comparison. Level of significance p=0.05.

One-Way ANOVA (Non-parametric)

Kruskal-Wallis	χ²	df	p
MS DV	8.02	2	0.018
MS IBA	5.26	2	0.072
MS W	0.985	2	0.611
MS ID	1.55	2	0.460

Table 5a. Non-parametric test for the 4 different treatments of Trial 2. Each p-value indicates the statistical significance of the detected differences within the treatment. Level of significance p=0.05.

Dwass-Steel-Critchlow-Fligner pairwise comparisons

	p1	p2	p3	p4
HA-26-PR - PH-BC2-91	0.129	0.793	0.805	0.515
HA-26-PR - HA-98	0.043	0.214	0.609	0.974
PH-BC2-91 - HA-98	0.489	0.060	0.911	0.608

Table 5b. DSCF comparisons of genotype pairs for each treament of Trial 2. p1 refers to treatment MS DV, p2 to MS IBA, p3 to MS W and p4 to MS ID. The p-values indicate the statistically significant differences between each pairwise comparison. Level of significance p=0.05.

One-Way ANOVA (Non-parametric)

Kruskal-Wallis	χ²	df	p
HA-98	-	-	-
NO-SU-12	-	-	-
PH-BC2-91	26.6	4	<.001
HA-26-PR	10.1	4	0.039

Table 6a. Non-parametric test of the 4 genotypes of Trial 3. Each p-value indicates the statistical signficance of the detected differences within the genotype. Level of significance p=0.05.

Dwass-Steel-Critchlow-Fligner pairwise comparisons

	p1	p2	p3	p4
MS K - MS DV	-	-	<.001	0.030
MS K - MS IBA	-	-	0.891	0.619
MS K - MS W	-	-	0.984	0.221
MS K - MS ID	-	-	0.891	0.068

Table 6b. DSCF comparisons of treatments compared to control treatment MS K for each genotype of Trial 3. p1 refers to HA-98, p2 to NO-SU-12, p3 to PH-BC2-91 and p4 to HA-26-PR. The p-values indcate the stistically significant differences between each pairwise comparison. Level of significance p=0.05.

One-Way ANOVA (Non-parametric)

Kruskal-Wallis	χ²	df	p
MS DV	3.93	2	0.140
MS IBA	0.0742	1	0.785
MS W	2.33	1	0.127
MS ID	4.51	1	0.034

Table 7a. Non-parametric test for the 4 different treatments of Trial 3. Each p-value indicates the statistical

Dwass-Steel-Critchlow-Fligner pairwise comparisons

	p1	p2	p3	p4
NO-SU-12 - HA-26-PR	-	-	-	-
NO-SU-12 - PH-BC2-91	-	-	-	-
NO-SU-12 - HA-98	-	-	-	-
HA-26-PR - PH-BC2-91	0.662	0.785	0.127	0.034
HA-26-PR - HA-98	-	-	-	-
PH-BC2-91 - HA-98	-	-	-	-

Table 7b. DSCF comparisons of genotype pairs for each treament of Trial 3. p1 refers to treatment MS DV, p2 to MS IBA, p3 to MS W and

significance of the detected differences within the treatment. Level of significance p=0.05.

p4 to MS ID. The p-values indicate the statistically significant differences between each pairwise comparison. Level of significance p=0.05.

One-Way ANOVA (Non-parametric)

Kruskal-Wallis	χ²	df	p
PH-BC2-91	17.8	4	0.001
HA-26-PR	4.12	4	0.390

Table 8a. Non-parametric test of the 2 genotypes of Trial 4. Each p-value indicates the statistical signficance of the detected differences within the genotype. Level of significance p=0.05.

Dwass-Steel-Critchlow-Fligner pairwise comparisons

	p1	p2
MS K - **MS DV**	0.053	0.995
MS K - **MS IBA**	0.053	0.774
MS K - **MS W**	0.161	0.989
MS K - **MS ID**	1.000	0.989

Table 8b. Table 6b: DSCF comparisons of treatments compared to control treatment MS K for each genotype of Trial 4. p1 refers to PH-BC2-91 and p2 to HA-26-PR. The p-values indcate the stistically significant differences between each pairwise comparison. Level of significance p=0.05.

One-Way ANOVA (Non-parametric)

Kruskal-Wallis	χ²	df	p
MS DV	1.90	1	0.168
MS IBA	4.43	1	0.035
MS W	0.00	1	1.000
MS ID	5.07	1	0.024

Table 9a. Non-parametric test for the 4 different treatments of Trial 4. Each p-value indicates the statistical significance of the detected differences within the treatment. Level of significance p=0.05.

Dwass-Steel-Critchlow-Fligner pairwise comparisons

	p1	p2	p3	p4
HA-26-PR - **PH-BC2-91**	0.168	0.035	1.000	0.024

Table 9b. DSCF comparisons of genotype pairs for each treament of Trial 4. p1 refers to treatment MS DV, p2 to MS IBA, p3 to MS W and p4 to MS ID. The p-values indicate the statistically significant differences between each pairwise comparison. Level of significance p=0.05.

One-Way ANOVA (Non-parametric)

Kruskal-Wallis	χ²	df	p
PH-BC2-91	33.7	4	<.001
HA-26-PR	8.55	3	0.036

Table 10a. Non-parametric test of the 2 genotypes of Trial 5. Each p-value indicates the statistical signficance of the detected differences within the genotype. Level of significance p=0.05.

Dwass-Steel-Critchlow-Fligner pairwise comparisons

	p1	p2
MS K - **MS DV**	<.001	0.139
MS K - **MS IBA**	0.161	0.980
MS K - **MS W**	0.447	-
MS K - **MS ID**	0.817	1.000

Table 10b. Table 6b: DSCF comparisons of treatments compared to control treatment MS K for each genotype of Trial 4. p1 refers to PH-BC2-91 and p2 to HA-26-PR. The p-values indcate the stistically significant differences between each pairwise comparison. Level of significance p=0.05.

One-Way ANOVA (Non-parametric)

Kruskal-Wallis	χ^2	df	p
MS DV	0.575	1	0.448
MS IBA	-	-	-
MS W	0.00	1	1.000
MS ID	1.15	1	0.284

Table 11a. Non-parametric test for the 4 different treatments of Trial54. Each p-value indicates the statistical significance of the detected differences within the treatment. Level of significance p=0.05.

Dwass-Steel-Critchlow-Fligner pairwise comparisons

	p1	p2	p3	p4
HA-26-PR - **PH-BC2-91**	0.449	-	1.000	0.283

Table 11b. DSCF comparisons of genotype pairs for each treament of Trial 5. p1 refers to treatment MS DV, p2 to MS IBA, p3 to MS W and p4 to MS ID. The p-values indicate the statistically significant differences between each pairwise comparison. Level of significance p=0.05.

		ROOT FORMATION % OF GENOTYPE PER MEDIUM									
		MS K		**MS DV**		**MS IBA**		**MS W**		**MS ID**	
		%	**St. Dev.**	%	**St. Dev.**	%	**St. Dev.**	%	**St. Dev.**	%	**St. Dev.**
Trial 1	**NO-SU-12**	**75.0%**	44.7%	**87.5%**	34.2%	**93.8%**	25.0%	**87.5%**	34.2%	**62.5%**	50.0%
	HA-26-PR	**56.3%**	51.2%	**93.8%**	25.0%	**68.8%**	47.9%	**56.3%**	51.2%	**93.8%**	25.0%
	PH-BC2-91	**68.8%**	47.9%	**93.8%**	25.0%	**81.3%**	40.3%	**87.5%**	34.2%	**62.5%**	50.0%
	HA-98	**25.0%**	44.7%	**93.8%**	25.0%	**56.3%**	51.2%	**93.8%**	25.0%	**37.5%**	50.0%
Trial 2	**NO-SU-12**	**25.0%**	50.0%	**-**	-	**-**	-	**0.0%**	0.0%	**50.0%**	53.5%
	HA-26-PR	**31.3%**	47.9%	**56.3%**	51.2%	**50.0%**	52.2%	**35.7%**	49.7%	**56.3%**	51.2%
	PH-BC2-91	**37.5%**	50.0%	**87.5%**	34.2%	**62.5%**	50.0%	**25.0%**	44.7%	**75.0%**	44.7%
	HA-98	**0.0%**	0.0%	**100.0%**	0.0%	**12.5%**	35.4%	**18.2%**	40.5%	**50.0%**	57.7%
Trial 3	**NO-SU-12**	**-**	-	**-**	-	**-**	-	**-**	-	**-**	-
	HA-26-PR	**0.0%**	0.0%	**66.7%**	49.2%	**25.0%**	50.0%	**50.0%**	57.7%	**58.3%**	51.5%
	PH-BC2-91	**7.1%**	26.7%	**81.3%**	40.3%	**18.8%**	40.3%	**0.0%**	0.0%	**18.8%**	40.3%
	HA-98	**-**	-	**100.0%**	0.0%	**-**	-	**-**	-	**-**	-
Trial 4	**PH-BC2-91**	**50.0%**	51.6%	**93.8%**	25.0%	**93.8%**	25.0%	**87.5%**	34.2%	**50.0%**	0.0%
	HA-26-PR	**81.3%**	40.3%	**75.0%**	45.2%	**62.5%**	50.0%	**87.5%**	34.2%	**87.5%**	50.0%
Trial 5	**PH-BC2-91**	**0.0%**	0.0%	**87.5%**	34.2%	**31.3%**	47.9%	**18.8%**	40.3%	**8.3%**	28.9%
	HA-26-PR	**25.0%**	45.2%	**66.7%**	49.2%	**-**	-	**18.8%**	40.3%	**25.0%**	45.2%

Table 12. Root regeneration percentages and Standard Deviation of each different genotype in each specific treatment among all 5 trials. NO-SU-12, HA-26-PR, PH-BC2-91, and HA-98 represent the 4 different genotypes tested. MS, DV, IBA, W and ID represent the treatments MS K, MS DV, MS IBA, MS W and MS ID respectively.

Razvoj efikasnog sistema za regeneraciju suncokreta: Ispitivanje različitih metoda za *in vitro* ožiljavanje četiri genotipa

Kimon Ionas · Emilija Bulić · Aleksandra Radanović · Ankica Kondić-Špika · Katarina Cankar · Dragana Miladinović

Sažetak: Suncokret predstavlja jednu od najznačajnijih uljanih kultura. Usled sve izraženijih klimatskih promena, pronalaženje efikasnih i pouzdanih načina za povećanje otpornosti biljaka na abiotički stres postaje imperativ. Genetička modifikacija predstavlja izuzetno precizan, ekonomičan i pouzdan način za unapređenje otpornosti suncokreta na abiotički stres, a naročito na sušu. Budući da suncokret spada u vrste koje se teško regenerišu i transformišu (rekancitrantnost), definisanje visokoefikasnog protokola je od ključne važnosti za uspeh genetičkih modifikacija. Cilj ovog istraživanja bio je procena kapaciteta za regeneraciju korena kod četiri različita genotipa suncokreta na pet hranljivih podloga, kako bi se identifikovala kombinacija genotipa i podloge sa najvišom stopom ožiljavanja. Procenat ožiljavanja varirao je u zavisnosti od ogleda (od 0% do 100%), pri čemu je najkonzistentnije formiranje korena učestalo beleženo kod genotipova PH-BC2-91 i HA-26-PR. Statistički značajno veće stope regeneracije korena ostvarene su na MS DV podlozi u poređenju sa ostale četiri korišćene podloge (oko 90% na MS DV u odnosu na približno 50% na ostalim podlogama). Ovi rezultati dodatno potvrđuju složenu interakciju između genotipa i hranljive podloge. Naša studija pruža pouzdanu osnovu za uspostavljanje efikasnog protokola za regeneraciju korena suncokreta i utire put primeni biotehnoloških pristupa u oplemenjivanju na otpornost prema abiotičkom stresu.

Ključne reči: indol-3-buterna kiselina, IBA, in vitro, podloga za ožiljavanje, regeneracija korena, suncokret, sušni stres