2013; GRA(1), 1-8

Effects of an agitated high dilution (10^{-30} parts by weight) of gibberellic acid on wheat stalk growth – a repetition study. Prestimulation of wheat seedlings with gibberellic acid (10^{-2} parts by weight) followed by application of the agitated high dilution

Helmut Graunke, Wolfgang Matzer, Waltraud Scherer-Pongratz, Harald Lothaller, P.C. Endler
Interuniversity College for Health and Development Graz / Castle of Seggau, Austria
college@inter-uni.net

ABSTRACT

Background

In previous multicentre studies, the influence of a homeopathic ultra-high dilution of gibberellic acid on wheat growth was scrutinized. Data showed that this test dilution slowed down stalk growth when experiments were performed in the autumn season.

Objective

The aim of this work was (a) to repeat this initial experiment and (b) test the hypothesis that pretreatment of grains with a high concentration of gibberellic acid would enhance the growth-inhibiting effect of the ultra-high dilution of the plant hormone.

Methods

Grains of winter wheat (*Triticum aestivum*, 500 per group) were either treated water ("W0") for control or were pretreated with an aqueous solution of (non-agitated) gibberellic acid 10^{-2} parts by weight (Ge-2) prior to further treatment. Grains were then observed under the influence of extremely diluted gibberellic acid (10^{-30} parts by weight) prepared by stepwise dilution and agitation according to a protocol derived from homeopathy ("G30x"). Inert water ("W0") and analogously prepared water ("W30x") were used for control. Seedlings were allowed to develop under standardized conditions for 7 days; plants were harvested and stalk lengths were measured.

Results

Mean stalk length in the W0/G30x group was 41.2±15.7 mm, i.e. 12.5% smaller than it was in the W0/W30x group (46.9±15.5 mm) and 12.9% smaller than it was in the W0/W0 group (47.4±16.8). Both differences are statistically significant ($p < 0.001$) and both are associated with a large effect size ($d = 1.2$ in both cases).

Stalk length in the Ge-2/ groups were practically alike (44.2 mm) ($p > 0.05$).

Concerning pretreatment under study, Ge-2 yielded less growth than W0. This outcome was modulated by the application of G30x in that the inhibition obtained with G30x as compared to W30x was greater when no pretreatment had been applied (Figure 5).

Conclusion

The hypothesis (a) that G30x would exert an inhibiting effect on stalk growth was accepted. This hypothesis followed from the authors' previous studies [1,2,3]). With regard to (b) it was observed that pretreatment with Ge-2, i.e. gibberellic acid 10^{-2} parts by weight (molarity $5x10^{-5}$) did not lead to a stronger effect of treatment with G30x, on the contrary, stalk growth under G30x was not different to stalk growth under W30x.

INTRODUCTION

In previous multicentre studies, the influence of a homeopathic ultra-high dilution of gibberellic acid on wheat stalk growth was scrutinized [1,2,3]. Data showed that this test dilution slowed down stalk growth when experiments were performed in the autumn season. Furthermore, the hypothesis was tested that pretreatment of grains with high concentrations of gibberellic acid would enhance the growth-inhibiting effect of the ultra-high
dilution of the plant hormone [3]. Grains of winter wheat (*Triticum aestivum*, 500 or 1000 per group) were pretreated with (non-agitated) gibberellic acid 10^{-5}, 10^{-4} and 10^{-3} parts by weight (Ge-5, Ge-4, Ge-3) or with water ("W0") for control prior to further treatment. Grains were then observed under the influence of extremely diluted gibberellic acid (10^{-30} parts by weigth) prepared by stepwise dilution and agitation according to a protocol derived from homeopathy ("G30x"). Analogously prepared water was used for control ("W30x").

Seedlings were allowed to develop under standardized conditions for 7 days; plants were harvested and stalk lengths were measured. Of the four pretreatment variants under study, Ge-3 yielded most growth, followed by Ge-4 , Ge-5 and finally W0. This outcome was modulated by the application of G30x in that the inhibition obtained with G30x as compared to W30x was the greater the lower the pretreatment concentration of G had been.
Figure 1 shows the results in terms of relative stalk length. Grain numbers were initially 1,000 each for Ge-4/W30x and Ge-4/G30x, and 500 for each of the other treatment groups, but only germinated grains were included in the results.

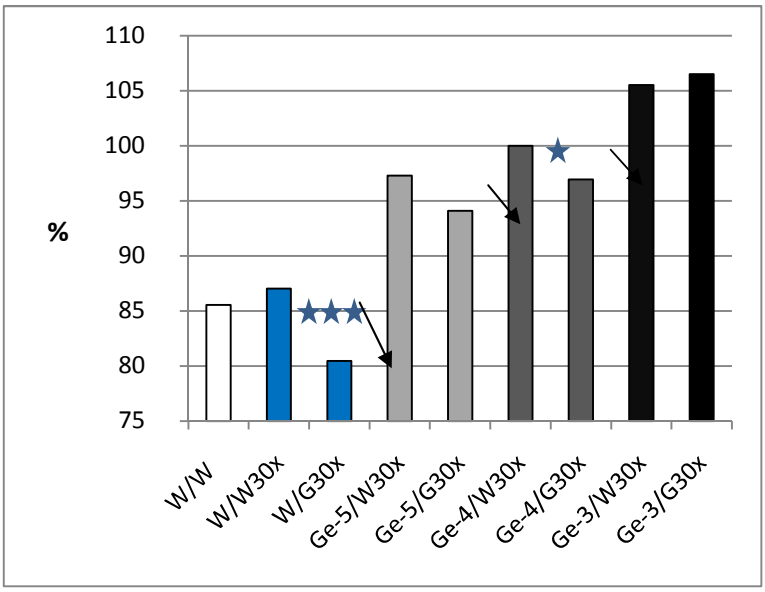

*Figure 1: Results from studies 1 and 2 showing relative stalk length by treatment group with Ge-4/W30x normalized to 100%. "W" is synonymous for "W0". N per group = 500, except for Ge-4/W30x (N = 1000) and Ge-4/G30x (N = 1000). ***, p < 0.001; *, p < 0.01. P-values refer to pairwise comparison of W30x versus G30x groups. For further p-values, see [3]. For further explanations see text.*

The hypothesis that pretreatment of grains with high concentrations of gibberellic acid would enhance the growth inhibiting effect of G30x had to be rejected. Rather, G30x slowed down stalk growth most

in the W0 group with $p < 0.001$, only moderately in the Ge-5 and Ge-4 group and not at all in the Ge-3 group.

The aim of the study presented here was

a) to have the effect of G30x on wheat stalk length reinvestigated, and
b) to investigate the effect of G30x after prestimulation of wheat seedlings by molecular doses of gibberellic acid (10^{-2} parts by weight).

Our hypothesis (a) was that G30x would exert an inhibiting effect on stalk growth (this hypothesis follows from the authors' previous studies [1,2,3]). No hypothesis was stated for (b).

METHODS

Experiments were documented in accordance with the recommendations of the K. and V. Carstens Foundation, Essen for good fundamental research documentation in homeopathy [4].

Plants

Experiments were performed on wheat (*Triticum aestivum*, Capo variety, procured from Fritz organic farming, Ottendorf, Styria, Austria) grain grown without herbicides or pesticides (harvest 2012). Around 10% of the grains were ruptured and around 10% were distorted, and these were all removed prior to the experiments.

Researchers, sites and dates

Experiments were performed by HG together with WS (autumn 2012) at the laboratory of the Interuniversity College in Weiz near Graz. The project was supervised by CE (for initials, see list of authors).

Laboratory conditions

All glass bottles and fastenings as well as the plastic pipettes used for the dilution process were disposable products. All dishes and covering glass vessels as well as the glass pipettes used for administration of the probes were heat sterilized and rinsed twice with double distilled water prior to use. Seedling development took place in complete darkness at a temperature of (21.0 ± 0.2)°C.

Preparation of solutions

Treatment substances

Grains were observed under the influence of extremely diluted agitated gibberellic acid with analogously prepared water serving as control. The treatment substance was prepared by stepwise dilution and succussion using a method derived from homeopathy and inspired in detail by Baumgartner [5,6]. The degree of dilution was set to 10^{-30} so as to exceed Avogadro's limit of theoretical zero-molarity (10^{-24}). Gibberellic acid was chosen as the active agent on account of the crucial role of its derivatives in normal plant development.

For preparation of the treatment substance, 0.017 g of gibberellic acid (Sigma-Aldrich company, art. no. 36575) were added to 9 ml of water, and the resulting 5 millimolar solution (substance "1") was gently swung (not "agitated") in a glass bottle for one minute (= "mother substance"). Then, using a disposable pipette (Brand company, Transferpette 100□□, 1 ml of the mother substance was added to 9 ml of double distilled water in a 20 ml brown glass bottle (Heiland company, art. no. 380020), and the product was agitated vigorously according to a standardized protocol: The bottle was manually banged 30 times against an elastic surface at intervals of approximately 0.5s to create mechanical shocks (= "G2x"). In a total of 30 steps of dilution 1:10 and 29 steps of agitation (as agitation was omitted at the first dilution step), the test substance "G30x" was thus prepared. Starting from the 28th step, quantities larger than 1ml were added to the tenfold amount of double distilled water in order to

prepare a sufficient quantity of test substance. Larger brown glass bottles (each of which was filled half with the liquid) were used for these last steps (29x: 250 ml, 30x: 500 ml). A new glass bottle was used for each dilution step.
Analogously prepared solvent was used for control ("W30x") to ensure that any solute contents of the glass wall would be equally present both in verum 30x and control 30x and the content of solute oxygen would also be alike. Thus, any difference in growth observed between verum- and control-treated seedlings would be attributable to the presence or absence of gibberellic acid in the mother substance.
Untreated water (=W0) served as an additional control.

Pretreatment substances
As can be seen in Table 1, second row, grains were pretreated with inert water control (for blinding purposes) or with gibberellic acid 10^{-2} parts by weight (molarity $5x10^{-5}$), respectively.

Treatment	W0	W30x	G30x	W30x	G30x
Pretreatment	W0	W0	W0	Ge-2	Ge-2

Table 1: Substances used for pre treatment and treatment. For explanations, see text.

All test substances (for treatment and pretreatment) were prepared by WS. Substances were applied one day after preparation.

System performance controls
Previous experiments had shown that differential treatment with W30x or with water that has not undergone any preparation process at all (W0, negative control) produces no differences in stalk length measured after one week (W30x: 50 ± 22 mm; W0: 50 ± 21 mm). The number of grains per group in these earlier experiments was 2,000, and temperature was (21.5 ± 1.0)°C.
Previous analysis of wheat growth under treatment with inert water control with the same spatial arrangement of dishes and plants as in the present study had shown a high degree of statistical homogeneity within dishes.

Independent probe coding
Control and verum were encoded by an independent authority. All probes were applied blindly; codes were broken only after the data had been calculated.

Data base
5 treatment groups of 500 grains were observed in the study. There were 20 grains per dish, i.e. 25 dishes per treatment group.

Placement of grains
Grains were arranged circularly in glass dishes (diameter 11 cm), each containing 1 layer of filter paper (Whatman, cellulose, 90 mm, sort 2), with the germination furrow facing down (Figure 2).

Figure 2: Placement of grains (from [2]).

Exposure to probes

For *pretreatment*, 2 ml of W0 or Ge-2, respectively, were added to each dish using a disposable 5 ml pipette and pipetting ball (VWR company, art. no. 612-1328 and 612-1947), and grains were left to soak for 4 hours. For *treatment*, 3 ml of G30x, W30x or W0 were added to the dishes, respectively. Dishes were then covered with 1000 ml glass vessels (up-side down beakers) and dishes and covers were wrapped in aluminium foil (Figure 3).

Figure 3: Seedling cultivation in beakers (from [2]).

Beakers were placed in alternating rows according to a random procedure (stratified randomization).

Figure 4: Stalk growth. From [1].

Observed development (endpoints)

Germination and stalk length (Figure 4) were observed after 7 days according to a standard protocol [2]. Stalks were cut off by one person and measured by naked eye on a mm scale by another person. The person performing the measurements knew neither how stalks had been treated (see blinding procedure above) nor what their blind code was. Any possibility of an assignment bias was thus ruled out. Results were recorded by a third person. Dishes were harvested in the same sequence as they had been prepared.

Data evaluation

Differences in germination rate were evaluated at the end of the experiment, i.e. after seven days, by entering the number of germinated and non-germinated seedlings of each treatment group and its corresponding control group in four-field tables according to the chi square test.

Stalk length was determined in terms of the arithmetic mean per dish and its S.D. and evaluated by one way analysis of variance. P-values were corrected for multiple testing. Effect sizes (Cohen's d, standardized difference of means = absolute difference between means of 2 groups, divided by S.D.) were also calculated. An effect size is considered small when > 0.2, medium when > 0.5 and large when > 0.8.

Data were evaluated blindly, i.e. the statistician (HL) was not aware of the meaning of the codes used. Codes were broken only after calculation of results.

RESULTS

Germination rates after 7 days were practically alike (ca. 98%) in all groups ($p > 0.05$).

Tables 2 and Figures 5 give an overview of the results on stalk growth.

Table 2 shows the stalk length data of studies 1 and 2 both separately and pooled.

Treatment	W0	W30x	G30x	W30x	G30x
Pretreatment	W0	W0	W0	Ge-2	Ge-2
mean±SD	47.4±6.7	46.9±5.1	41.2±3.9	44.2±3.6	44.2±6.5
%	*100*	*99.1*	*87.1*	*93.3*	*93.3*

Table 2: Absolute stalk length (mean±S.D. at dish level; mm) by treatment group. Percentages refer to the W0/W0 group. For further explanations, see text.

(A) Mean stalk length in the W0/G30x group was 41.2±15.7 mm, i.e. 12.5% smaller than it was in the W0/W30x group (46.9±15.5 mm) and 12.9% smaller than it was in the W0/W0 group (47.4±16.8). Both differences are statistically significant ($p < 0.001$) and both are associated with a large effect size ($d = 1.2$ in both cases).

(B) Stalk length in the Ge-2/ groups were practically alike (44.2 mm) ($p > 0.05$).

Figure 5 shows the results in terms of relative stalk length.

*Figure 5: Relative stalk length by treatment group with W0/W0 normalized to 100% ("W" is synonymous for "W0"). N per group = 500. ***, p < 0.001; the P-value refers to pair wise comparison of W30x versus G30x groups. Only germinated grains were considered. For further explanations see text.*

Concerning pretreatment under study, Ge-2 yielded less growth than W0. This outcome was modulated by the application of G30x in that the inhibition obtained with G30x as compared to W30x was greater when no pretreatment had been applied (Figure 5).

CONCLUSION

The hypothesis (a) that G30x would exert an inhibiting effect on stalk growth was accepted. This hypothesis followed from the authors' previous studies [1,2,3]). With regard to (b) it was observed that pretreatment with Ge-2, i.e. gibberellic acid 10^{-2} parts by weight (molarity $5x10^{-5}$) did not lead to a stronger effect of treatment with G30x, on the contrary, stalk growth under G30x was not different to stalk growth under W30x.

Although the wheat model was inspired by effects observed in intoxication / detoxification (or more precise: isopathic detoxication) experiments, and although pretreatment by hyperstimulation with the hormone at a molecular dose level enhanced biological development (stalk growth), the results obtained are suggestive neither of an intoxication / detoxication mechanism, nor of an "inversion effect" of the homeopathic dilution with regard to the effect of the mother substance.

An interesting way to carry these investigations further might be to pretreat wheat seedlings with gibberellin antagonists prior to treatment with extremely diluted agitated gibberellic acid or to expose them to growth inhibiting factors.

ANNOTATION

With the agreement of the authors and the editor of [3], without being a reprint, this paper uses descriptive elements of [3] for the presentation of its new data.

REFERENCES

[1] Pfleger A., Hofäcker J., Scherer-Pongratz W., Lothaller H., Reich C., Endler P.C. (2011) The effect of extremely diluted agitated gibberellic acid (10e-30) on wheat seedling development – a two researcher study. Complementary Therapies in Medicine 19, 164-169.

[2] Endler P.C., Matzer W., Reich C., Reischl T., Hartmann A.M., Thieves K., Pfleger A., Hofäcker J., Lothaller H., Scherer-Pongratz W.: Seasonal variation of the effect of extremely diluted agitated gibberellic acid (10e-30) on wheat stalk growth – a multi researcher study. The Scientific World Journal 2011; 11: 1667-1678 13.

[3] Hribar-Marko S., Graunke H., Scherer-Pongratz W., Lothaller H., Endler P.C. Prestimulation of wheat seedling development with gibberellic acid (10e-3, 10e-4 and 10e-5) followed by application of an agitated high dilution (10e-30) of the same hormone. IJHDR 2013; 12(42): 26-39.

[4] Stock-Schröer B., Albrecht H., Betti L., Endler P.C., Linde K., Lüdtke R., Musial F., van Wijk R., Witt C., Baumgartner S. (2009) Reporting experiments in homeopathic basic research (REHBaR) – A detailed guideline for authors. Homeopathy 98, 287-298.

[5] Baumgartner S., Thurneysen A., Heusser P. (2004) Growth stimulation of dwarf peas (Pisum sativum L.) through homeopathic potencies of plant growth substances. Res Compl Med / Forsch Komplementärmed 11, 281-292.

[6] Baumgartner S., Shah D., Schaller J., Kämpfer U., Thurneysen A., Heusser P. (2008) Reproducibility of dwarf pea shoot growth stimulation by homeopathic potencies of gibberellic acid, Complem Ther Med 16(4), 183-191.