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Reproducibility of dwarf pea shoot growth stimulation by homeopathic potencies of gibberellic acid

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Summary
Objectives: Investigation of the conditions for reproducibility of dwarf pea shoot growth stimulation through homeopathic potencies of gibberellic acid.
Methods: 4 batches of pea seed (*Pisum sativum* L. cv. Früher Zwerg; harvests from 1997, 1998, 1999, and 2000) were tested regarding their reaction to gibberellic acid 17x and 18x (compared to unsuccussed and succussed water (1x) as controls) in 8 independent randomized and blinded experiments. Pea seed was immersed for 24 h in watery solutions of homeopathic potencies or controls, and cultivated under controlled laboratory conditions. Pea shoot length was measured after 14 days. Two systematic negative control experiments assessed the stability of the experimental set-up.
Results: The systematic negative control experiments yielded no significant effects and confirmed the stability of the experimental set-up. 2 out of 4 seed batches reacted to the homeopathic treatment ($p < 0.05$). Seed batch 1997 showed a reproducible reaction to gibberellic acid 17x (shoot length stimulation of +11.2%, $p = 0.007$), and seed batch 1998 showed a significant varying response (increase/decrease). Seed batch 1997 differed from the other 3 batches by an increased glucose and fructose content, and reduced 1000 kernel weight. Meta-analysis with data of earlier experiments is in accordance with the results of the present experimental series.
Conclusions: We identified 'seed quality' as a possible trigger factor for successful reproducibility in homeopathic basic research. Premature harvesting as a possible key factor for responsiveness of dwarf peas to homeopathic potencies of gibberellic acid is our current working hypothesis to be tested in future experiments.

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Introduction

Specific effects of homeopathic remedies (as used in homeopathy and anthroposophical medicine) have been observed in several randomized clinical trials.[1–4] Incompatibility of homeopathic remedy production standards[5] with current standard pharmacological theories[6] can lead to the conclusion that these clinical results must be false-positive artefacts.[7] The opposite consequence — the actual state of knowledge of physics and chemistry is incomplete — is also discussed.[8] However, any such conclusion would require convincing empirical evidence. The latter may arise from clinical as well as from preclinical research.

Basic research into homeopathic potentization can be grouped into four main areas: animal research, plant bioassays (including plant-pathogen interactions), *in vitro* models (fungi, bacteria, cell cultures and biochemical tests), and physicochemical research. Recent reviews[9–12] of these four areas all ended up with a similar conclusion: there is some evidence for substance specific effects of homeopathic potencies, but only very few models could be independently reproduced. Several reproductions failed[13–16] or resulted in inverted or altered effects.[17–23] Even internal reproduction seems to be a non-trivial task,[24–27] giving rise to speculations about irreproducibility as a possibly inherent feature of homeopathic potency effects.[28,29]

The aim of the present study was to investigate conditions for internal reproducibility of the dwarf pea model.[26] We had observed that gibberellic acid 17x induced a reproducible shoot growth increase, which albeit seemed to weaken in the course of the experiments. In this follow-up study we wanted to answer two questions: 1. Can another experimenter reproduce the effects observed? 2. Has the factor 'seed batch' a relevant influence on the results?

Methods

General experimental design

We replaced the person in charge for potentization, seed treatment, plant care and final measurement (S.B. by D.S.) and investigated the effects of homeopathic potencies of gibberellic acid on four batches of dwarf pea seed (harvests from 1997, 1998, 1999, and 2000). We tested the effect of water, succussed water (1x), gibberellic acid 17x and 18x on pea shoot growth in 8 independent experiments, all fully randomized and blinded. In every experiment, three seed batches were used (2000, 1999, 1998 or 1997 — the latter two alternating). A typical experiment involving homeopathic potencies comprises 200 seeds (4 parameters × 50 seeds) per pea batch (harvest).

In order to investigate the stability of the experimental set-up, we conducted two systematic negative control experiments. These are full-size experiments with 800 seeds each (50 seeds × 4 harvests × 4 treatment parameters), using unsuccussed water as identical cultivation parameter.

Furthermore, seed batches were characterized regarding major chemical constituents (sugar, starch, amino acids) and 1000 kernel weight.

Preparation of homeopathic potencies and control solutions

Full details concerning the preparation of the investigated solutions (homeopathic potencies of gibberellic acid and controls) are given in an earlier publication.[26] In short, gibberellic acid (Sigma, Switzerland) was dissolved and succussed in acetone (Merck, Switzerland) to obtain 50 mM gibberellic acid (1x). All further potency levels (2x—18x) were obtained by potentization in distilled water according to the multiple glass method (Hahnemann—H—potencies, decimal steps). In contrast to the former protocol,[26] potentization vessels were reused in successive experiments after thorough rinsing once with deionized and twice with distilled water. Control solutions were unsuccussed and succussed (1x) water, according to the reasoning published elsewhere.[30] Potencies and controls — using the same batch of distilled water — were prepared freshly for each experiment. The test solutions were coded with a letter code by another person not being involved in the experiments.

Plant cultivation

Plant cultivation also was described in detail earlier.[26] In contrast to the former protocol,[26] 15 ± 0.2 g of dwarf pea seed (*Pisum sativum* L., cv. Früher Zwerg) were immersed in 100 ml homeopathic or control solution for 24 h in duplicate. Seed of four different harvests (1997, 1998, 1999, 2000) was used. Due to a limited residual amount, seed of 1997 and 1998 was used in alternating experiments. 2×25 well-swollen grains were selected at random and planted into 2×5 pots, filled with a standard cultivation substrate, a 1:1 mixture (v/v) of TKS1 (peat, Floragard, Germany) and Vermex F (expanded vermiculite, Vermica AG, Switzerland). Pots were watered with tap water from beneath (400 ml per pot during the entire cultivation). Plants were grown in artificial lighting of 60 ± 10 μmol/m^2 s photosynthetic active radiation (PAR) in a 14 h/10 h light/dark cycle at controlled temperature (24 ± 2 °C). After two weeks, plants were harvested and shoot length was measured by stretching the plants on a sheet of millimeter graph paper. Trays were cleaned by hand with a brush and hot tap water. In contrast to the former protocol,[26] pots were cleaned in a dishwasher. Both pots and trays were reused in random assignment in subsequent experiments.

Analysis of pea seed carbohydrates

Dry pea seeds (approx. 5 g) were ground to a fine powder with a mechanical grinder. Samples of 100 mg were extracted four times with 1 ml 80% aqueous ethanol to remove soluble sugars. Insoluble material containing the starch was resuspended in 1 ml water and homogenised further with a pestle and mortar to obtain a homogeneous suspension. Aliquots of 200 μl were autoclaved for 20 min at 121 °C to solubilise the starch and 200 μl of Na Acetate, pH 4.8 containing 10 units of α-amylase and 1.25 units of amyloglucosidase was added to digest the starch to glucose (37 °C, 16 h). Liberated glucose was measured as described elsewhere.[31] Control samples treated in the same way but incubated without enzymes contained no glucose. To

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determine soluble sugars, the four 80% ethanol extracts for each sample were combined and 2 ml evaporated to a small volume (400 μl on average) under an air stream. Glucose and fructose content were determined spectrophotometrically as described previously.[31,32] To measure sucrose, 100 μl of the evaporated soluble extract was added to 100 μl of Na Acetate, pH 4.8 containing 150 units of β-fructosidase to hydrolyse sucrose to glucose and fructose (37 °C, 2 h). Total glucose and fructose was measured and the sucrose content calculated by subtracting the values for the free hexose sugars measured in the undigested extract. All samples were coded and analyzed in 6-fold replication.

Amino acid analysis of pea seed

Dry pea seeds (approx. 5 g) were ground to a fine powder with a mechanical grinder. Samples were hydrolysed in the gas phase with 6M hydrochloric acid containing 0.1% (by volume) phenol for 24 h at 115 °C under N_2 vacuum according to Chang and Knecht.[33] The liberated amino acids were reacted with phenylisothiocyanate and the resulting phenylthiocarbamoyl amino acids were analyzed by RP-HPLC on a Nova Pak ODS column (3.9 mm × 150 mm, 4 μm; Waters) in a Dionex Summit liquid chromatograph with an automatic injection system according to Bidlingmeyer et al.[34] and Cohen and Strydom.[35] All samples were coded and analyzed in triplicate.

Data evaluation and statistical analysis

All data analysis was performed with the statistics software 'Statistica 4.1 for Mac' (Statsoft, Inc., Tulsa, OK 74104, USA).

Effects of the treatments on mean shoot length were statistically analyzed by a 2-way analysis of variance (ANOVA), comprising treatment and experiment number (date) as the two independent variables (factors) and pea shoot length

Table 1 Pea shoot length (mean ± standard deviation [mm]) for all seed batches and treatment groups in eight independent experiments

Experiment N°	Seed batch	Treatment			
		Unsuccussed water	Successed water	GA_3 17x	GA_3 18x
33	1997	79.5 ± 38.9	71.7 ± 21.4	85.9 ± 28.1	73.3 ± 25.5
34		–	–	–	–
35		66.6 ± 22.7	67.7 ± 21.0	86.5 ± 25.9	74.6 ± 31.5
36		–	–	–	–
37		81.0 ± 27.0	74.6 ± 24.4	71.4 ± 23.4	61.6 ± 28.4
38		–	–	–	–
39		67.4 ± 30.4	69.8 ± 31.4	77.8 ± 19.4	73.7 ± 22.0
40		–	–	–	–
33	1998	–	–	–	–
34		88.7 ± 23.6	85.0 ± 24.6	83.1 ± 25.3	86.7 ± 24.0
35		–	–	–	–
36		83.9 ± 22.3	94.1 ± 21.6	79.7 ± 23.0	86.5 ± 24.7
37		–	–	–	–
38		76.6 ± 21.6	76.3 ± 24.3	90.7 ± 22.6	81.3 ± 28.4
39		–	–	–	–
40		81.2 ± 26.5	75.4 ± 16.4	73.2 ± 25.5	73.3 ± 19.6
33	1999	108.3 ± 23.6	104.6 ± 24.6	103.9 ± 25.3	107.3 ± 24.0
34		103.8 ± 22.3	105.6 ± 21.6	108.9 ± 23.0	107.4 ± 24.7
35		104.5 ± 21.6	105.1 ± 24.3	104.0 ± 22.6	101.9 ± 28.4
36		103.8 ± 26.5	107.6 ± 16.4	100.4 ± 25.5	102.5 ± 19.6
37		97.4 ± 23.6	97.5 ± 24.6	96.1 ± 25.3	103.4 ± 24.0
38		93.2 ± 22.3	94.8 ± 21.6	90.3 ± 23.0	96.7 ± 24.7
39		100.4 ± 21.6	91.9 ± 24.3	97.9 ± 22.6	100.0 ± 28.4
40		95.6 ± 26.5	99.1 ± 16.4	96.5 ± 25.5	95.3 ± 19.6
33	2000	105.5 ± 23.2	104.9 ± 25.5	98.6 ± 24.4	102.0 ± 21.8
34		105.3 ± 21.9	101.6 ± 20.8	109.0 ± 27.3	108.7 ± 21.1
35		99.5 ± 24.4	96.3 ± 19.4	98.8 ± 19.3	101.3 ± 22.6
36		103.3 ± 28.0	101 ± 19.6	99.6 ± 28.4	101.1 ± 23.8
37		100.3 ± 19.3	99.4 ± 22.1	94.8 ± 19.5	107.0 ± 22.0
38		92.8 ± 22.0	92.1 ± 20.4	94.1 ± 23.5	101.0 ± 21.9
39		100.2 ± 21.1	96.9 ± 22.3	93.9 ± 17.9	97.4 ± 21.9
40		93.8 ± 23.6	95.7 ± 19.6	95.7 ± 22.2	93.6 ± 24.7

Experiment N° = internal experiment numbering.

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[mm] as dependent variable. All experiments were evaluated separately for the four seed batches (due to the — from a statistical point of view — 'incomplete' experimental design). If not otherwise stated, *p*-values refer to analysis of variance *F*-tests. Planned comparisons were evaluated with the LSD test only if the preceding *F*-test was significant ($p < 0.05$). This procedure (protected Fisher's LSD) gives a good safeguard against type I error without being too conservative, i.e. it also gives good security against type II error.[36]

Betti et al. recently proposed variability (instead of mean values) as a new target in homeopathic basic research.[27,37] We therefore estimated variability in our experiments as follows. Variability *within* experiments was assessed by first normalizing the length data of all plants treated with gibberellic acid 17x to the combined water control (CWC, defined as 100%), separately for every experiment and every seed batch. Data were then pooled and the standard deviation calculated. Variability *between* experiments was estimated by computing the absolute length means for the parameters combined water control and GA_3 17x for each experiment, and by calculation of the standard deviation of the corresponding group means.

Results

Systematic negative control experiments

No significant differences could be detected between the four identical treatment parameters or in any interaction between treatment and experiment number ($p > 0.25$ in all cases). We thus conclude that the experimental system was stable and did not produce false-positive results for identical treatment parameters.

Experiments with homeopathic preparations of gibberellic acid

Descriptive statistics for all groups are given in Table 1.

No statistically significant differences were found between unsuccussed and succussed water (the two controls used) for any seed batch, neither as main effect nor as interaction with the experiment number ($p > 0.1$ in all cases, *F*-test, Table 2, analysis 1: succussion effect). Correspondingly, it is possible to pool all data from the plants treated with unsuccussed and succussed water into a combined water control group.

Significant treatment effects for the homeopathic preparations were found for seed batch 1997 and 1998, but not for seed batch 1999 and 2000 (Table 2, analysis 2: potency effect).

Dwarf pea seed of harvest 1997 reacted reproducibly to the homeopathic preparations (significant main effect, no interaction with date of experiment). Compared to the combined water control, gibberellic acid 17x increased pea shoot growth by +11.2% ($p = 0.007$), whilst gibberellic acid 18x did not show comparable effects (−2.1%, $p = 0.62$).

Seed of harvest 1998 exhibited a varying response to the homeopathic treatment (no significant main effect, but a significant interaction with the date of experiment). Compared to the combined water control, gibberellic acid 17x decreased pea shoot growth by −10.5% ($p = 0.047$) in experiment N° 36 and increased shoot growth by +18.6% ($p = 0.002$) in N° 38. Gibberellic acid 18x did not induce any significant effects.

Meta-analysis of all experiments with gibberellic acid 17x

Data of the present experiments were pooled with those published earlier.[26]

Table 2 Results of the global statistical analysis (ANOVA *F*-test) of the 8 experiments newly reported in this publication (N° 33—40)

Seed batch	ANOVA factors	Analysis 1: succussion effect		Analysis 2: potency effect	
		Parameters	*p*-Value*	Parameters	*p*-Value*
1997	1: Experiment N°	33, 35, 37, 39	0.1090	33, 35, 37, 39	0.3752
	2: Treatment	W, SW	0.4692	W, SW, GA_3 17x, GA_3 18x	**0.0251**
	1/2: Interaction		0.6873		0.1105
1998	1: Experiment N°	34, 36, 38, 40	0.0005	34, 36, 38, 40	0.0002
	2: Treatment	W, SW	0.9637	W, SW, GA_3 17x, GA_3 18x	0.9745
	1/2: Interaction		0.1089		**0.0102**
1999	1: Experiment N°	33—40	0.0000	33—40	0.0000
	2: Treatment	W, SW	0.9395	W, SW, GA_3 17x, GA_3 18x	0.5934
	1/2: Interaction		0.4691		0.7078
2000	1: Experiment N°	33—40	0.0007	33—40	0.0000
	2: Treatment	W, SW	0.3152	W, SW, GA_3 17x, GA_3 18x	0.1202
	1/2: Interaction		0.9909		0.7097

Analysis 1 compares unsuccussed water (W) and succussed water (SW) only; analysis 2 includes unsuccussed water (W), succussed water (SW), gibberellic acid 17x and gibberellic acid 18x.

* Significant ($p < 0.05$) treatment effects (main effects or interactions) are printed bold.

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Table 3 Results of the global statistical analysis (ANOVA *F*-test) for the pooled data set (all new experiments (N° 33–40) and those published earlier (No. 2, 8–17, published in[26]))

Seed batch	ANOVA factors	Analysis 1: succussion effect		Analysis 2: potency effect	
		Parameters†	*p*-Value*	Parameters†	*p*-Value*
1997	1: Experiment N°	2, 33, 35, 37, 39	0.0000	2, 12, 14, 33, 35, 37, 39	0.0000
	2: Treatment	W, SW	0.3466	CWC, GA_3 17x	**0.0005**
	1/2: Interaction		0.8253		0.0557
1998	1: Experiment N°	8–11, 16, 34, 36, 38, 40	0.0000	8–11, 13, 16, 34, 36, 38, 40	0.0000
	2: Treatment	W, SW	0.6871	CWC, GA_3 17x	0.3801
	1/2: Interaction		0.2171		**0.0112**
1999	1: Experiment N°	17, 33–40	0.0000	15, 17, 33–40	0.0000
	2: Treatment	W, SW	0.7042	CWC, GA_3 17x	0.7697
	1/2: Interaction		0.5266		0.6188
2000	1: Experiment N°	33–40	0.0007	33–40	0.0000
	2: Treatment	W, SW	0.3152	CWC, GA_3 17x	0.3679
	1/2: Interaction		0.9909		0.3413

Analysis 1 compares unsuccussed water (W) and succussed water (SW); analysis 2 comprises the combined water control (CWC) and gibberellic acid 17x.

† Experiments No. 12–15 did not include a succussed water control.

* Significant ($p < 0.05$) treatment effects (main effects or interactions) are printed bold.

No statistically significant differences were found between unsuccussed and succussed water for any seed batch, neither as main effect nor as interaction with experiment number ($p > 0.2$ in all cases, *F*-test, Table 3, analysis 1). It is thus possible to pool all data from the plants treated with unsuccussed and succussed water into a combined water control group.

Again, significant treatment effects for gibberellic acid 17x were found for 1997 and 1998 seed batches only (Table 3, analysis 2). Dwarf pea seed of harvest 1997 reacted reproducibly (significant main effect, no interaction with date of experiment): compared to the combined water control, GA_3 17x increased pea shoot growth by +10.2% ($p = 0.0004$, Fig. 1). Seed of harvest 1998 exhibited a varying response to the homeopathic treatment (no significant main effect, but a significant interaction with experiment number, Fig. 2). Regarding single experiments, No. 36 and 38 showed significant effects of gibberellic acid 17x (*p*-values see above).

For seed batch 1997 and 1999, variability *within* experiments was somewhat lower for the gibberellic acid 17x treated groups (Table 4); for seed batch 1998 and 2000, this tendency was inversed. Regarding variability *between* experiments, the gibberellic acid 17x treated groups all

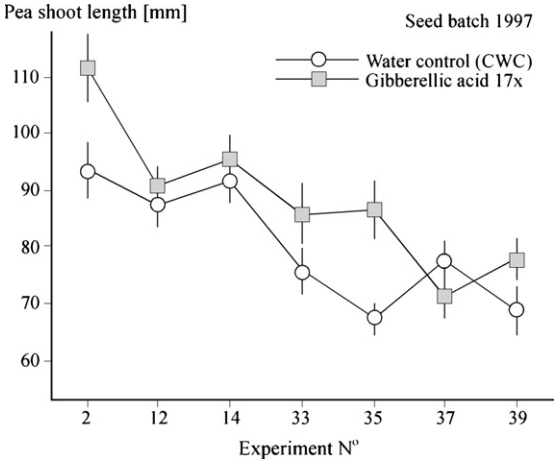

Figure 1 Pea shoot length (mean ± standard error [mm]) of all experiments involving seed batch 1997, treated with either water or gibberellic acid 17x. Data of the water control group (combined water control, CWC) were pooled from the plants treated with either unsuccussed or succussed water. Data of the experiments No. 2, 12, and 14 have been published earlier.[26]

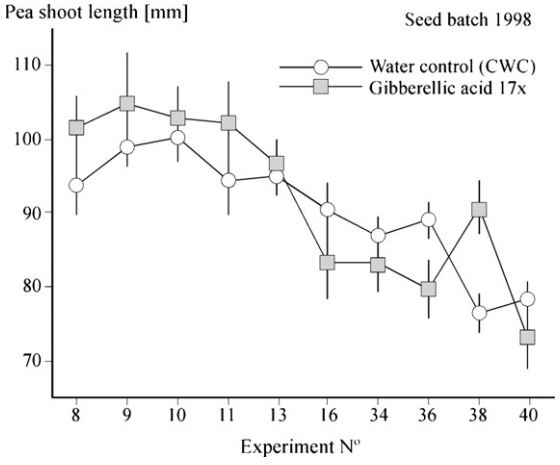

Figure 2 Pea shoot length (mean ± standard error [mm]) of all experiments with seed batch 1998, treated with either water or gibberellic acid 17x. Data of the water control group (combined water control, CWC) were pooled from the plants treated with either unsuccussed or succussed water. Data of the experiments No. 8–11, 13, and 16 have already been published earlier.[26]

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Table 4 Assessment of variability *within* experiments

Seed batch	Combined water control (CWC)		GA_3 17x	
	S.D.† [%]	*n**	S.D.† [%]	*n**
1997	35.34	360	32.43	221
1998	25.92	608	28.57	326
1999	20.65	894	20.28	469
2000	22.17	780	23.30	391

Calculation: see 'Data evaluation and statistical analysis.' Included experiments: see Table 3 (analysis 2).
† S.D. = standard deviation [%].
* *n* = number of plants measured.

showed a higher inter-experimental variability (on the average +27%, details see Table 5).

Analysis of seed batch properties

Seed batch 1997 differs from the other three by a substantially larger standard deviation (Table 4, column CWC). Seed batch 1998 lies between batch 1997 and 1999/2000.

The four seed batches used were analyzed regarding several chemical compounds (Table 6). Whilst there were no major differences in single as well as total amino acid and starch content, the concentration of glucose and fructose of seed batch 1997 was elevated by about 100%. In addition, 1000 kernel weight was decreased by about 10%.

Discussion

Results of the present experimental series are clear-cut. Two out of four seed batches did react to gibberellic acid 17x. Seed batch 1997 showed a reproducible stimulating effect of +11.2%, whilst seed batch 1998 showed a varying response to the homeopathic treatment (increase/decrease). Seed of harvest 1999 or 2000 did not react — within the limits given by statistical power.

There are many possible reasons for the differential reaction of the four seed batches to GA_3 17x. Age of the seeds can be excluded since there was no relation between the effect of GA_3 17x and the increasing age of seed batch 1997 in the course of the experiments. Other possible factors relate to influences during cultivation of the plants which yielded the seed batches used in the present experiments, such as climate, water supply, soil, fertilization, plant diseases, insect attack, and harvesting date. Interestingly, seed batch 1997 differs from the other harvests by a more or less doubled glucose and fructose content and by a reduced 1000 kernel weight. This observation is compatible with the hypothesis of insufficient ripening.[38,39] We thus put forward premature harvest as hypothesis for dwarf pea seed responsiveness to treatment with gibberellic acid 17x. This hypothesis can be tested by production of corresponding seeds. If verified, this would mean that peas have to be in a suboptimal physiological state to show a reaction to GA_3 17x.

Table 5 Assessment of variability *between* experiments

Combined water control (CWC)		GA_3 17x	
S.D.† [mm]	*n**	S.D.† [mm]	*n**
10.73	7	12.92	7
8.00	10	11.37	10
6.99	10	9.02	10
4.32	8	4.96	8

Calculation: see 'Data evaluation and statistical analysis.' Included experiments: see Table 3 (analysis 2).
† S.D. = standard deviation [mm].
* *n* = number of experiments.

The increased standard deviation of the 1997 and 1998 harvests (about 65% and 24%, respectively, compared to the 1999 and 2000 harvests) may be partially due to lowered vitality of the plants because of premature harvest. Increasing age of the seed during the course of the experiments may also be a reason for the higher standard deviation, but most probably not for the increase in glucose and fructose content.

The initial reasoning to choose dwarf peas to investigate effects of homeopathic potencies relied on the fact that dwarf peas bear a mutation stopping the gibberellic acid synthesis pathway leading to low endogenous levels of GA_1 and subsequent dwarf growth. Given the strong differences between the seed batches investigated, this mutation is not the only condition for peas to respond to potentized gibberellic acid. It may even be not necessary at all, since Scherr et al.[40] observed effects of homeopathic potencies of gibberellic acid on the growth of duckweed (*Lemna gibba* L.), which is not a gibberellic acid deficient mutant. A comparison of the reaction of normal and dwarf peas to potentized gibberellic acid would allow to test the role of the dwarf growth mutation.

According to the statistical analysis, seed batch 1998 did show a significant interaction with the date of the experiment, i.e. a varying response to the homeopathic treatment (increase/decrease), whilst seed batch 1997 did not. This means that the reaction of pea plants of batch 1998 to gibberellic acid 17x was modulated by some factor associated with the date of the experiment. At present we have no idea about the nature of this factor. We are quite confident that the observed variability in the reaction of seed batch 1998 is no experimental artifact, i.e. a false-positive result, because the systematic negative control experiments as well as the comparison of the two water controls indicate that the experimental system was stable. More data are needed, however, to further document and investigate any such effect modulation. Due to the larger standard deviation of seed batch 1997, the statistical power of the analysis is reduced compared to the other seed batches. It thus may be that the reaction of seed batch 1997 also was modulated by analogous factors (Fig. 1), but any such effect did not manifest in the statistical analysis.

Gibberellic acid 18x did not induce any significant effects in the present experiments. This confirms the notion that adjacent potency levels may strongly differ in their biological effects[26,40,41] as first documented by Kolisko.[42]

The meta-analysis, including all earlier data with the same model,[26] essentially yields the same results as the analysis of the new data set alone. We thus presume that the experimenters either did not substantially influence the

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Table 6 Seed batch properties: 1000 Kernel weight and analytical data (sugars, starch, and total amino acids), mean ± standard deviation

	Unit	Seed batch			
		1997	1998	1999	2000
1000 kernel weight	[g]	224.5 ± 7.1	250.9 ± 8.9	258.7 ± 5.5	259.7 ± 3.9
Sucrose	[mg/g DW]	32.2 ± 0.2	27.7 ± 2.0	28.9 ± 0.9	27.5 ± 1.7
Glucose	[μg/g DW]	85.9 ± 4.1	48.6 ± 9.0	42.5 ± 4.0	55.0 ± 2.2
Fructose	[μg/g DW]	88.4 ± 8.6	27.0 ± 9.6	19.9 ± 1.9	31.7 ± 2.4
Starch	[mg/g DW]	436.7 ± 29.9	464.8 ± 33.4	431.1 ± 4.2	426.7 ± 17.3
Alanine	[μmol/g DW]	848 ± 93	745 ± 206	835 ± 89	830 ± 93
Arginine	[μmol/g DW]	904 ± 75	784 ± 240	1101 ± 139	1118 ± 118
Aspartic acid	[μmol/g DW]	1412 ± 137	1228 ± 326	1345 ± 111	1386 ± 154
Cystine	[μmol/g DW]	50 ± 4	42 ± 11	33 ± 1	40 ± 6
Glutamic acid	[μmol/g DW]	1880 ± 186	1636 ± 465	1761 ± 189	1856 ± 210
Glycine	[μmol/g DW]	1018 ± 100	873 ± 260	987 ± 124	1006 ± 87
Histidine	[μmol/g DW]	259 ± 25	217 ± 69	244 ± 23	256 ± 26
Isoleucine	[μmol/g DW]	510 ± 56	432 ± 138	474 ± 63	498 ± 53
Leucine	[μmol/g DW]	897 ± 88	774 ± 214	888 ± 123	906 ± 84
Lysine	[μmol/g DW]	811 ± 83	704 ± 195	796 ± 83	821 ± 88
Methionine	[μmol/g DW]	107 ± 13	98 ± 33	74 ± 10	95 ± 15
Phenylalanine	[μmol/g DW]	483 ± 55	417 ± 126	468 ± 53	474 ± 52
Proline	[μmol/g DW]	594 ± 61	510 ± 146	588 ± 77	591 ± 50
Serine	[μmol/g DW]	731 ± 70	634 ± 171	722 ± 96	737 ± 71
Threonine	[μmol/g DW]	467 ± 49	401 ± 116	446 ± 64	456 ± 45
Tyrosine	[μmol/g DW]	302 ± 22	262 ± 89	282 ± 45	295 ± 37
Valine	[μmol/g DW]	637 ± 60	536 ± 169	587 ± 70	622 ± 69
Sum amino acids	[μmol/g DW]	11910 ± 1156	10293 ± 2969	11631 ± 1359	11987 ± 1225

Samples were analyzed in triplicate (6-fold for sugars and starch). DW = dry weight.

effect of gibberellic acid 17x on pea shoot growth or did not differ regarding their influence. Conclusive evidence regarding possible experimenter effects can only be gained from experiments carried out by different persons in parallel. Since each experiment was conducted with freshly potentized gibberellic acid, all experiments can be regarded as independent. Taken together with the systematic negative control experiments performed, we conclude that there is good evidence for a specific biological effect of gibberellic acid 17x on pea shoot growth of seed batch 1997.

The tentative 'decline effect' of gibberellic acid 17x on pea shoot growth during the course of the first set of experiments[26] could not be confirmed. Its (not significant) appearance was most probably due to the change from seed batch 1997 to seed batches 1998 and 1999 during these experiments.

Though gibberellic acid 17x (5×10^{-18} M) is not an ultramolecular concentration, its effect on pea shoot growth is far beyond the end of the classical dose-response curve situated at GA_3 6x (5×10^{-7} M).[26] As discussed elsewhere in detail,[26] the effect observed cannot be designated as hormetic effect. One reason is the lacking effect inversion (from negative to positive) in our experiments.

We did not observe any effect of water succussion on pea shoot growth. Thus the present pea model does not seem to be sensitive to the unspecific effects associated with succussion, such as air suspension and dissolution, pH alterations, radical formation, and enhanced ion release from potentization vessel walls. This further validates the stability of the system, and the reliability and specificity of the effects of gibberellic acid 17x.

To summarize, we observed statistically significant effects of gibberellic acid 17x on pea shoot growth for two out of four seed batches. We thus identified 'seed quality' as a possible factor leading to problems with reproducibility in homeopathic basic research. Our present working hypothesis to be tested in future experiments is premature harvest as a possible trigger factor for responsiveness of dwarf peas to homeopathically potentized gibberellic acid.

Conflict of interest

There are no conflicts of interest.

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