THE EFFECT OF HOMOEOPATHICALLY PREPARED DILUTIONS OF GIBBERELLIC ACID ON THE GERMINATION OF BARLEY SEED (*HORDEUM VULGARE* L.) AS MEASURED BY A GERMINATION INDEX.

by

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Dissertation submitted in partial compliance with the requirements for the Master's Degree in Technology: Homoeopathy in the Department of Homoeopathy at the Technikon Natal, Durban.

I, Karen Him Lok, do hereby declare that this dissertation represents my own work both in concept and execution.

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ACKNOWLEDGEMENTS

I wish to express my appreciation to the following persons for assisting and guiding me in the preparation of this dissertation:

Dr Brigitte Hamman, supervisor. Department of Natural Sciences, University of Pretoria.

Dr Russell Hopkins, co-supervisor. Department of Homoeopathy, Natal Technikon.

Dr Ashley Ross, Head of Department, Homoeopathy, Technikon Natal.

Dr Gwen Koning. Department of Natural Sciences, University of Pretoria.

In addition I would like to thank:

Professor H. A. Van de Venter for his assistance and for allowing me the use of laboratory facilities at the Department of Natural Sciences, University of Pretoria.

Richard Him Lok, for his encouragement, assistance and use of computer facilities.

Dr Richard Steele, for his advice and assistance.

The barley seeds used in this study were kindly donated by Sensako.

The homoeopathic preparations were kindly produced by Natura.

Special thanks to my mother and father for all their strength and guidance.

Thank you Dr Ross and Dr Hopkins, for your contribution to my education and training.

To my colleagues, Angela Cason, Troy Davies, Natasha Louw, Justin Middelborough and David Naudè, I am grateful for all your encouragement and support.

ABSTRACT

The potentisation process during which homoeopathic preparations are produced, raises the concern that these remedies have a placebo effect, since they contain no active molecule of the substance used to prepare them (in ultra high dilutions) by the time they are administered to a patient. Plant models therefore offer a more direct method of examining the efficacy of homoeopathically prepared solutions. This study investigated the effects of homoeopathic preparations of gibberellic acid on the germination of barley seeds (*Hordeum vulgare* L.) as measured by a germination index.

The effects of GA_3 at potencies of 4cH, 15cH, 30cH and 200cH on germination rate and seedling development were tested on barley seeds of high-, medium- and low- vigour. The index used comprised the rate at which seeds germinated, which was the time it took for 50 percent of those seeds that did germinate, to germinate (i.e. T_{50}); seedling development experiments as assessed by taking final germination counts 7 days after start of imbibition, shoot and root lengths, and seedling dry mass.

Biological activity of homoeopathically prepared GA_3 at 15cH was evident in medium vigour seeds, since this treatment resulted in significantly long root development. High-vigour barley seeds imbibed in GA_3 (0.5 g L^{-1}) were found to germinate the fastest of all treatment groups, and high-vigour seeds imbibed in homoeopathically prepared GA_3 at 4cH, 30cH and 200cH, germinated faster than those seeds not receiving GA_3 in any form (the control). Amongst high-vigour seeds, prior imbibition with homoeopathically

prepared GA_3 at 4cH, 15cH, 30cH and 200cH solutions stimulated the subsequent development of larger seedlings than those imbibed in GA_3.

Both the germination rate and seedling development experiments therefore demonstrated biological activity of homoeopathically prepared GA_3 at 4cH, 30cH and 200cH, even in the presence of exogenous GA_3, and in so doing, demonstrated biphasic effects of GA_3 in various concentrations.

The barley seed germination plant model successfully demonstrated biological effects of homoeopathically prepared gibberellic acid in various potencies i.e. 4cH, 15cH, 30cH and 200cH. This model also demonstrated that certain effects not produced by a substance in its crude (unpotentised) state are able to only manifest when the substance is used in its homoeopathically-potentised state.

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AOSA	-	Association of Official Seed Analysts
cH	-	centesimal Hahnemannian
GA_3	-	gibberellic acid
HGA_3	-	homoeopathically-prepared gibberellic acid
ISTA	-	International Seed Testing Association
x	-	decimal potency, denotes dilutions processed in steps of 1:10

DEFINITION OF TERMS

Absolute Alcohol

Anhydrous alcohol, theoretically 100% alcohol. In practice it is dehydrated alcohol, with a minimal admixture of water, at most 1%. Its specific gravity is 0.792 (Gaier 1991:23).

AOSA

The initials of the Association of Official Seed Analysts, the organisation of state and federal seed analysts of the United States and Canada (Copeland and McDonald, 1989:376).

Arndt-Schultz Law

This law states that every stimulus on a living cell elicits an activity, which is inversely proportional to the intensity of the stimulus (Oberbaum & Cambar, 1994:6).

Attenuation

Dilution, thinning. Reduction or weakening (Steadman's Pocket Medical Dictionary, 1983)

Avogadro's limit

Avogadro's Number (6.02254×10^{23} mol^{-1}) (Majerus, 1991), is the constant number of molecules in a mole of any substance. The Avogadro limit is reached at 12CH or 23DH

and in homoeopathic dilutions higher than these not a single molecule of the original substance or mother tincture is expected to remain (Gaier, 1991: 47).

Biphasic Action

Hahnemann referred to drugs when he described the biphasic action as 'Most medicines have more than one kind of effect - the direct one at the beginning, passing gradually into the second (indirect after-effect). The latter state is generally exactly opposite to the former'. Therefore biphasic action is defined as the observed action of certain substances (commonly medicinal substances), where the first direct, coercive action gradually changes into the second reactive effect, which is more or less opposite to the first (Gaier, 1991:185).

Dilution

Rarefaction; the act of reducing the concentration of a solution or a non-fluid mixture, or the resultant solution or non-fluid mixture, proper. Occasionally used erroneously as a synonym for (liquid) potency or dynamisation, but correctly used to describe (liquid) non-homoeopathic medicines that have, in the course of their preparation, undergone dynamisation procedures resembling those characteristic of homoeopharmaceutics, as in Organotherapeutic or Anthroposophic remedies, for example (Gaier, 1991:128).

Dynamisation

Dynamisation is different to simple dilution due to the periodic dilutions, separated by a number of shakes (succussions) (Zacharais and Zacharais, 1997). A synonym of dynamisation is potentisation. See potentisation.

Embryo

The generative part of a seed that develops from the union of the egg cell and sperm cell and during germination becomes the young plant (Copeland and McDonald, 1989:380).

Endosperm

The tissue of seeds that develops from sexual fusion of the polar nuclei of the ovule and the second male sperm cell. It provides nutrition for the developing, growing embryo (Copeland and McDonald, 1989:380).

Ethanol (C_2H_5OH)

This reacts with acids to form esters and with alkali metals to form alcoholates. It is used in homoeopathy as a vehicle, solvent and preservative, relative to internal medicines, and as a disinfectant, externally (Gaier 1991:23).

Germination

The resumption of active growth by the embryo culminating in the development of a young plant from the seed (Copeland and McDonald, 1995:381).

Gibberellic acids

A group of growth promoting substances first discovered in the *Gibberella spp.* They regulate many growth responses and appear to be a universal component of seeds as well as other plant parts (Copeland and McDonald, 1989:382).

Homoeopathy

Self-consistent scientific system of medicinal therapy. It is based on the observed biological fact that a diseased deviation from an organism's bioenergetic mean, within reversible limits, can predictably be restored to normal by specially prepared medicinal stimuli. These need only be administered in small doses, or more often in sub-physiological concentrations, owing to an altered receptivity of tissue to such stimuli in disease, provided always (a) that in healthy organisms the medicinal agents chosen would produce symptoms and clinical features like those of the disease and, (b) that obstacles to cure have been removed (Gaier, 1991:272).

Hormesis

Biphasic medicinal dose/response action; describes the reversed biological effects in various ranges of concentrations of the same medicinal agent. This phenomenon refers to the biphasic dose response first described by Hahnemann (Gaier, 1991:275).

Hormesis is a term first proposed by Southam and Ehrlich (1943) to describe "a stimulatory effect of subinhibitory concentrations of any toxic substance on any

organism". The Arndt-Schultz law formed the basis of the hormesis phenomenon (Schofield, 1984).

ISTA

The initials of the International Seed Testing Association (Copeland and McDonald, 1989:383).

Law of Stimuli

This law is formulated as: Minute stimuli encourage life activity, medium to strong stimuli tend to impede it, and very strong stimuli to stop or destroy it (Gaier, 1991:265).

Pharmacopoeia

Authoritative reference work containing monographs of medicines and other therapeutic agents, specifications for the sources of, and standards for the strength and purity of, base substances and mother tinctures, formulae and methods of preparation of these substances and their derivative properties, as well as descriptions of processes for the testing of starting materials (Gaier, 1991:398).

Placebo

In homoeopathic practice, it refers to a non-medicated substance, that is relatively inert pharmacodynamically, sometimes administered to allow a previous remedy a prolonged period of action without undue medicinal interference, or to allow for the observation of a patient for a period without homoeopathic medication in order to arrive at the similimum,

or, perhaps, to contrast the effects of relative non-medication in controlled experiments with those of medication in two comparable groups of patients (Gaier, 1991:426).

Plant Hormone

A chemical substance that is produced in one part of a plant and used in minute quantities to induce a growth response in another part (Copeland and McDonald, 1989:382).

Potency

The especially produced capability in a medicine to effect a dynamic stimulus in the appropriate patient (Gaier, 1991:432).

Potency also refers to a number appearing after the name of the homoeopathic substance, indicating the number of times the substance has been diluted and succussed or triturated.

Symbols used to signify potencies: the letter "D" or "x" i.e. decimal potency denotes Dilutions processed in steps of 1:10. The letter "C" or "c" i.e. centesimal potency denotes dilutions processed in steps of 1:100 (Gaier, 1991:432).

Potentisation

Preparation of Homoeopathic Dilutions (remedies); Dynamisation; imparting (along serial dilutions) the pharmacological message of the original substance (i.e. creating a template of the active principle) by means of trituration or succussion (Gaier, 1991:441).

Process of stepwise dilution of a substance e.g. in water or water-alcohol mixture and input of exogene energy by agitation (e.g. succussing or vortexing) between the dilution

steps. Non-water-soluble substances are first triturated with lactose (Andersch and Endler 1994:221).

Seed Vigour

Those seed properties which determine the potential for rapid uniform emergence and development of normal seedlings under a wide range of field conditions as defined by the AOSA (Copeland and McDonald, 1989:392).

Seedling

A young plant grown from seed (Copeland and McDonald, 1989:389).

Solute

The dissolved substance in a solution (Gaier, 1991:521).

Solution

'Solution' refers to tinctures or the homogenous state of a liquid, resulting from the incorporation of extraneous substance(s), not of biological origin, whether liquid, gaseous or solid (Gaier, 1991:521).

Succussion

The action of shaking vigorously a liquid dilution of a homoeopathic medicine in its vial/bottle, where each stroke ends with a jolt. Usually effected by pounding the hand engaged in the shaking, against the palm of the opposite hand (Gaier, 1991:532).

Ultra high dilution, UHD, High Dilution

Homoeopathically-prepared dilution of infinitesimally small quantities of the original substance, in general diluted beyond 10^{-23} (Andersch and Endler, 1994).

Vital Force

'Elan vital', or the momentum of life, of philosopher Henri Louis Bergson (1859-1941); the 'Lebenskraft' of Hahnemann (Gaier, 1991:558). The vital force is an influence which directs all aspects of life in the organism. It adapts to environmental influences, it animates the emotional life of the individual, it provides thoughts and creativity, and it conducts spiritual inspiration. That aspect of the vital force which establishes balance in states of disease is called the "defense mechanism" (Vithoulkas 1990:59).

INTRODUCTION

Much of the study of homoeopathy has been based on its beneficial use with regard to the well being of the individual, as far as their health is concerned (Hahnemann, 1842). Human subjects have been used successfully to prove the validity of homoeopathy (Linde et al, 1997) and yet the scientific world remains sceptical of the evidence that homoeopathy, its remedies and its theories, do indeed work.

Using plant models to test the efficacy of homoeopathic preparations can demonstrate whether or not dilution principles and theories of homoeopathic preparations, especially their ability to contain and improve the structural information of the original substance, credibly have an effect without circumspect of mere suggestion. This would also apply to the question of whether the effect differs among potencies. The advantage of using a plant model is that there is little room to speculate a placebo response. Thus the study of homoeopathy within agricultural science would serve as a suitable means with which to demonstrate the efficacy of homoeopathic remedies and philosophy.

Homoeopathy has already been applied to experimental models within the context of agriculture (Steele, 1999; Hopkins, 1998; Betti et al, 1994, Bornoroni, 1991). Homoeopathic researchers successfully utilised various plant models with which to investigated the effect of ultra-high dilutions of different mineral compounds, chemicals, poisonous substances and plant hormones on plant growth (Schofield, 1984).

Barley seed production depends on the successful establishment of a crop in the field. In order for a barley plant to develop, the vital process of seed germination must first occur. As for all seeds the germination of high quality seed is less problematic than that of lower quality seed.

Gibberellic acid (GA_3) is a plant growth hormone originally discovered as the cause of a disease of rice that stimulated internode elongation. Endogenous gibberellins influence a wide variety of developmental processes, among them the control of various aspects of seed germination, including the loss of dormancy and the mobilisation of endosperm (Taiz and Zeiger, 1998:602). As a plant growth regulator, exogenously applied GA has been found to be of great use in the crop industry for stimulating germination in seeds which are otherwise slow to begin growth (Hill, 1980:65). GA_3 can successfully break dormancy in numerous species of seed and can also accelerate germination of non-dormant seeds (Bewely and Black, 1978:188).

In homoeopathy, the dynamisation process is used to prepare ultra-high-diluted remedies. Compared to straightforward dilution, dynamisation is technically different due to the periodic dilutions separated by a number of shakes or succussions (Zacharais and Zacharais, 1997). In each step of the dilution process, the concentration of molecules of the original substance decreases, but its information is not lost, and rather spreads over the whole of the more dilute solution. The shaking process promotes the spread of information and it results in a greater ability of the system to maintain the integral structure and function of the substance that was used in the first step of this process. The

information is better integrated and dynamically maintained in the more dilute solution (Resch and Guttmann, 1991).

Critics often argue that the success of homoeopathic treatment is merely a placebo effect. The school of psychology has tested the power of suggestion and has indeed found with the placebo effect that if someone is provided with a treatment, at times they improve. This improvement is due to a combination of the treatment plus the client's expectation of improving (Barlow and Durand, 1995:130).

If the original substance is soluble, one part is diluted with either ninety-nine parts of distilled water and/or alcohol and shaken vigorously (succussed). One part of the medicine is then further diluted and the process is repeated until the desired concentration is reached. Since the least amount of a substance in a solution is one molecule, a 30cH solution would have to have at least one molecule of the substance dissolved in a minimum of 10^{-57} g L^{-1} (Schofield, 1984). It was also calculated that one drop of starting material, which when mixed with a volume of diluent equivalent to the total volume of the earth, would not even equate to a concentration of 15CH (Majerus, 1991).

It is therefore improbable to many sceptics that these remedies have any effect at all and it is more believable that the homoeopath is prescribing water or water mixed with alcohol (Winston, 1999:451). Also, it may be difficult to believe that any one potency has a different effect to another potency. Critics find it inconceivable that the way in which these remedies are made could have any effects, much less beneficial effects, for

the patient, other than the mere suggestion that they are receiving a "treatment" (Winston, 1999:451).

Criticism of homoeopathy is leveled by both scientists well versed in homoeopathic literature and by the conventional physicians who are often not personally familiar with homoeopathic literature (Majerus, 1991). Schofield (1984) has critically reviewed homoeopathy and its potential role in agriculture. The conclusion is that there is little evidence to suggest that homoeopathy is effective although much experimental and clinical work has been done. This is not necessarily due to the fact that it is ineffective, instead it is due to bad design, execution, reporting or inability to repeat the experiments. In utilizing plant models to investigate the efficacy of homoeopathy, the conduction of well-designed, carefully controlled experiments is required if effectivity is to be conclusively demonstrated (Scofield, 1984).

This study investigated homoeopathically-prepared GA_3 in a range of potencies normally used by homoeopaths in both classical and clinical prescribing (Jouanny, 1993:83; Vithoulkas, 1990:217). The range represents dilutions above and below the Avogadro limit i.e. 4cH, 15cH, 30cH and 200cH potencies. The germination response of whole barley seeds to exogenously applied homoeopathically-prepared GA_3 was selected as a plant model which utilised high-, medium-, and low-vigour barley seeds. This study also assessed the potential of homoeopathically-prepared GA_3 as a viable alternative to GA_3 for enhancing germination performance of high-, medium- and low- quality barley seeds.

The implications of the study

Biological activity of homoeopathic preparations will be demonstrated without confounding factors (e.g. placebo effect).

Differential responses of dilutions above and below Avogadro's limit, will be investigated.

The benefits of the study

The main benefit of this study will be the demonstration of the efficacy of homoeopathic preparations using a suitable plant model. The materials are easily obtainable and relatively inexpensive. The equipment and apparatus are found normally in any laboratory and are easy to operate. Basic laboratory skills in conjunction with simple preparation are all that are required to execute this experiment. The entire experimental procedure can be completed within one month of full-time application.

Should homoeopathically-prepared GA_3 prove effective on the germination process of whole barley seeds, it will make a worthwhile contribution to the field of agro-homoeopathy.

Different levels of barley seed vigour were utilised in this experiment so as to compare the efficacy of homoeopathic preparations on seed with a range in quality. Germination performance of high quality seed will often differ to that of a lower quality seed lot. In

order to test the efficacy of homoeopathic preparations the inclusion of different levels of seed vigour were used. This research represents a unique contribution to the previous body of research that have investigated the effects of GA_3 in de-embryonated barley seeds, in that it investigated the effects of GA_3 in high- and ultra-high-dilutions in whole barley seeds of different vigour levels.

CHAPTER 1

LITERATURE REVIEW

Introduction

Criticism of homoeopathy is leveled by both scientists well versed in homoeopathic literature and by the conventional physicians who are often not personally familiar with homoeopathic literature (Majerus, 1991). Linde et al (1997) asked the question "are the clinical effects of homoeopathy placebo effects" in their meta–analysis of placebo controlled trials. Winston (1999:450) has quoted *The Lancet* which repeats the orthodox medical view "... What could be more absurd than the notion that a substance is therapeutically active in dilutions so great that a patient is unlikely to receive a single molecule of it?"

Many questions are raised with regard to the homoeopathic manner of preparing medicinal substances, especially the method of potentisation (Schulte, 1999). High dilutions reach Avogadro's limit and ultra-high dilutions are continued beyond this limit in the preparation of high potencies (Gaier, 1991:47). Diluting past Avogadro's limit means that not a single molecule of the original substance or mother tincture exists in solution any further (Gaier, 1991:47). It is this method of producing homoeopathic remedies that has raised much speculation over the probability that these remedies have a placebo effect by the time they are administered to a patient (Winston, 1999:452).

Homoeopathic Plant Models

Homoeopathy is an active form of medicine that reinforces the body's own healing powers and does not suppress them in the way antibiotics and cortisone do (Wolff, 1998:xi). A person's symptoms represent his body's best efforts to defend and heal itself, so in clinical practice, the main aim in homoeopathy is to stimulate the body's defense system (Ullman, 1992:2). The nervous, endocrine and immune systems together with the mind form an axis that regulates and controls the healthy functions of the body. A disturbance along this axis will cause a diseased state of the organism and it is along this axis that a homoeopathic medicine must act in order to effect a cure of the diseased state (Sankaran, 1991:37). Much speculation comes into play when humans are involved in homoeopathic experimentation, this is because a direct response cannot be observed and subjectivity is often involved concerning individual responses to the remedies. Subjectivity also arises due to the fact that each patient leads a different life and the environment in which they live differs for each person, therefore their degree of sensitivity to a homoeopathic remedy also varies greatly from person to person.

In contrast to humans, plants do not have a nervous system and immune system in order to obtain a response from the homoeopathic preparation. The ability of a plant to grow and survive depends on the vital force unique to that plant and its environment of growth. This is the vital force, the momentum of life (Gaier, 1991:558) that directs the plant to grow in the manner specific to that individual plant but the environment in which it grows is able to be controlled. The experimentation of homoeopathic preparations with the use of plant models offers a more direct method of investigation into the efficacy of

homoeopathic potentisation. Observing the effect of homoeopathic treatment on germination could demonstrate whether that treatment enhanced germination performance and a more direct response to homoeopathic treatment can be observed. These observations will allow us to examine the principles that define dose-dependant reverse effects of low and very low doses by homoeopathic standards (Gaier, 1991:265).

The use of plant models in homoeopathic experiments has allowed new ways in which to test the efficacy of homoeopathic preparations to develop. This has also generated a worldwide interest in a unique field of study called 'agro-homoeopathy' (Schofield, 1984). Agro-homoeopathy combines agricultural science and homoeopathic science. The significance of this work lies in that it offers homoeopathic treatment in agriculture and experimental proof that homoeopathic preparations and principles of homoeopathic potentisation have effect (Hopkins, 1998:2).

The stimulus for much of the work on the effect of homoeopathic potencies on the growth of plants was provided by the Koliskos' in 1978. The rationale behind the work was that the homoeopathic remedies replaced missing elements or deficiencies of them in the plant. In 1971, Pelikan and Unger did a detailed study of the effect of potentised silver nitrate ($AgNO_3$) on the growth of wheat (*Triticum durum*) seedlings. Forty growth trials repeated six times were involved. Each series exhibited the same type of curve, a three part growth curve rising from potencies 8x to 14x, falling to 16x and then rising again. This proved a suitable system in which to demonstrate the efficacy of homoeopathic preparations (Schofield, 1984).

Jones and Jenkins (1981) undertook a similar study using potencies of other substances as well as $AgNO_3$. Significant changes in growth were found with some of the potencies, and in the trials with $AgNO_3$ similar curves of changes in growth with increasing potency were obtained. It was however unfortunate that Jones and Jenkins used centesimal potencies whereas Pelikan and Unger used decimal potencies, because a comparison of the curves from these works would have been valuable (Schofield, 1984).

Jones and Jenkins (1983) compared the potential of yeast and wheat seedlings as models for testing homoeopathic remedies. They found that some potencies of *Pulsatilla nigricans* inhibited growth whilst others stimulated it, and the pattern of change was similar for both organisms and variations were claimed to be statistically significant (Schofield, 1984).

Hopkins (1998) studied the efficacy of homoeopathic medicines on germinability of different cultivars of *Lactuca sativa* L. (lettuce seeds) by applying different homoeopathic treatments (Sulphur, Nitric Acid and Camphor) utilising a range of different centesimal potencies to the whole seeds. It was demonstrated that certain of the homoeopathically-prepared medicines used, produced distinct biological effects. A consistently faster germination was observed in Camphor treated seed than Nitric Acid and Sulphur treated seeds. The results also provide evidence of the 3cH potency of the different treatments as having least effect with respect to germination promotion when compared to 9cH, 15cH and 30cH potencies.

The simple model proposed by Betti, L., Brizzi, M., Nani, D. and Peruzzi, M. (1994) in which homoeopathic potencies of *Arsenicum album* were used, examined wheat seed germination and concluded that the system was particularly suitable for studying homoeopathic potencies. Subsequent studies showed that the effect of high dilutions of *Arsenicum album* on wheat seedlings from seed poisoned with the same substance was limited to the stem. Significant recovery were shown, which increased with treatment time. The tendency to recover normal morphology was also observed in treated plants (Betti et al, 1997).

In a study to assess the effect of highly diluted solutions of $CaCO_3$ on the growth of oat (*Avena sativa*) coleoptiles stimulated by 100 μM of the growth hormone indoleacetic acid (IAA), it was found that specimens pretreated with 5cH $CaCO_3$ prior to the addition of IAA, exhibited a statistically significant increase in growth when compared to specimens treated with IAA alone. The study concluded that the action of dynamised $CaCO_3$, especially at 5cH, is synergistic and seems to increase the phyto-hormonal action of IAA (Bornoroni, 1991).

Pongratz and Endler (1994) showed enhanced growth of wheat seedlings under the influence of silver nitrate in the D24 and D26 potencies. They concluded that their study was proof of the reliability of a test system which had been previously quoted as a basic model for the research on homoeopathic drugs.

Scofield's conclusion is that there is little evidence to suggest that homoeopathy is effective even though much experimental and clinical work has been done. This is not

necessarily due to an inefficacy of the system which requires testing on a large enough scale, instead it is due to bad design, execution, reporting or inability to repeat the experiments. In utilising plant models to further investigate the efficacy of homoeopathy, the conduction of well-designed, carefully controlled experiments is required (Scofield, 1984).

Many of the papers Schofield considered in his critical review "Homoeopathy and its potential role in agriculture" (1984), did not make any statistical analysis of the data. Studies that discussed results that were obtained from data analysis, did not present the data in a way that allowed for independent assessment of the author's claims. Sample size and variance were not given. Statistical analysis was ignored in cases where results were negative and general conclusions were derived from data which would not have been supported by a proper analysis. In other papers precise methodological details are missing, which are important for repetition and critical assessment. In some trials criticised by Schofield in which a large range of potencies was tested and positive results were obtained, these have often been at potencies that are not normally used by homoeopaths.

Barley

Barley (*Hordeum vulgare* L.) is a monocotyledon of the Graminae family, the family of grasses. As one of the world's oldest domesticated crops (Matz, 1969:97), barley has many beneficial uses. It has been an important food plant during the development of agriculture where the grain was used as flour and to prepare fermented beverages. It is by far the most important cereal grain for malting and is used in the manufacture of beer

and whiskey. Approximately ¼ of the annual production, about 100 million bushels, is used for this purpose annually in the United States (Matz, 1969:99) and between 20% and 25% of the British and US barley crop respectively, is used in malting (Briggs, 1981:21). The remainder of the barley crop is utilised mainly in animal feedstuffs in which a high crude protein content is desirable.

Barley has been found to have medicinal uses. For example, it is used for the preparation of a decoction which is a nutritive and demulcent drink in febrile conditions and in catarrhal affections of the respiratory and urinary organs. Barley water prevents the formation of hard masses of curd in the stomach of infants (Grieve, 1931:84).

Plant Hormones

Finding commercially useful applications of our knowledge of endogenous plant growth substances has been a much slower process than one might have expected. One of the reasons for this is that the availability of some of the relevant chemicals was limited for a long time and many are still relatively expensive (Hill, 1980:64). Since 1980, an abundance of scientific research involving plant hormones can be found. These investigations involve gibberellic acid, abscisic acid and indole acetic acid to name a few. Plant scientists have been quite curious as to how the hormones control internal mechanisms of the plant with respect to growth. Few plant model studies however, have included the use of plant hormones and homoeopathic experimentation.

Plant hormones are substances produced in one part of the plant and transported to another where they elicit some type of response. Each plant hormone affects a wide variety of growth responses in the plant throughout its lifetime. These hormones interact with one another in both stimulatory and antagonistic ways (Ville et al, 1985:767).

The initiation of the metabolic pattern that occurs during germination involves the activation of specific enzymes at the proper time and regulation of their activity. Control is exercised by four classes of plant hormones: inhibitors such as abscisic acid, auxins that control root formation and growth; gibberellins which regulate protein synthesis and stem elongation; and cytokinins that control organ differentiation. Ethylene is also believed to have a control function in some plants. These plant hormones found naturally in seeds, have been extracted by scientists, applied to plant models in experiments, and their effect on growth has been observed (Bryant, 1985:40).

Gibberellic acid is a hormone first discovered in Japan in the 1930s from studies of diseased rice plants. These plants could not support themselves and eventually died from weakness and parasite damage. This was caused by the fungus *Gibberella fujikuroi* and the isolated active compound from the fungus was called gibberellin. Eighty-four gibberellanes have been discovered since 1990. All gibberellins are derivatives of the ent-gibberellane skeleton. The structure of this molecule is shown in Figure 1.1. (Salisbury and Ross, 1985:372). All gibberellins are acidic and are named GA for gibberellic acid with a different subscript to distinguish them. All gibberellins have either 19 or 20 carbon atoms grouped in either four or five ring systems. GA_3 is the first

highly active and longtime commercially available gibberellin and has historically been called gibberellic acid (Salisbury and Ross, 1985:372).

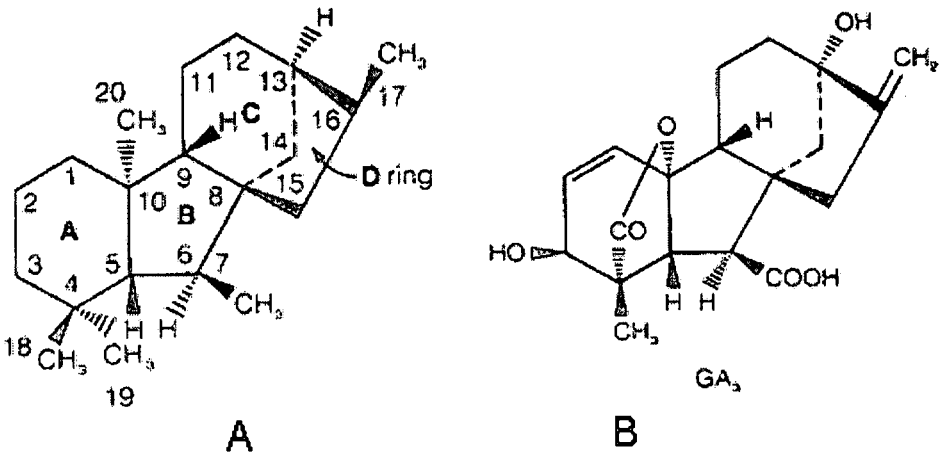

Fig 1.1 (A) Structure of the *ent*-gibberellane skeleton. (B) Structure of GA_3 (Salisbury & Ross, 1985:372).

Endogenous gibberellins influence a wide variety of developmental processes, including various aspects of seed germination, including the loss of dormancy and the mobilisation of endosperm. Gibberellins are known to stimulate internode elongation in a wide range of species (Taiz and Zeiger, 1998:602). Gibberellins are successful in breaking dormancy in numerous species of seed and also in accelerating germination of non-dormant seeds (Bewely and Black, 1978:188). Gibberellins are used commercially to increase the size of grape bunches, stimulate elongation of sugar cane and celery stalks, delaying senescence and maintaining firmer rinds thus preventing rind disorders of navel oranges. Gibberellins are used by some breweries to increase the rate of malting through the enhancing effect GA_3 has on starch digestion (Salisbury and Ross, 1985:381). It is also used for stimulating seeds which are otherwise slow to begin growth (Hill, 1980:64).

The availability of some endogenous plant growth substances and their relevant chemicals was limited for a long time and are relatively expensive. Many of the uses of gibberellins might otherwise be beneficial to crop growers to enhance crop production in appropriate circumstances (Hill, 1980:64).

The Role of GA_3 In Barley Seed Germination

In barley seed storage cells, mobilisation of foods and mineral elements are stimulated by gibberellin. The embryo of barley seeds is surrounded by food reserves (starch) in the metabolically inactive cells of the endosperm. The aleurone layer surrounds the endosperm. When the seed begins to germinate in response to increased moisture, the aleurone cells provide hydrolytic enzymes that digest the starch, proteins, RNA and cell wall materials present in the endosperm. The α–amylase enzyme is necessary for these digestion processes. The barley embryo provides gibberellin hormone to the aleurone layer and this stimulates the production of hydrolytic enzymes (such as α–amylase) in the endosperm where the digestion of food reserves follows. Reserve mineral elements become more readily available as a result of gibberellin action (Salisbury and Ross, 1985:381). The solubilised sugars, amino acids and other products are transported to the growing embryo but the embryo is responsible for the aleurone layer secretion of starch-degrading enzymes e.g. α–amylase. Fig. 1.2 is a simple diagram of the mobilisation of barley endosperm reserves by gibberellins and it illustrates the structure of barley seeds and the functions of various tissues during germination.

The germinating barley caryopsis has, for many years, provided the classical example for a hormonal action of a plant growth substance moving from the site of production to the target tissue where it triggers specific biochemical processes (Atzorn and Weiler, 1983). For instance, gibberellic acid can substitute for the embryo in stimulating starch degradation. When GA_3 is applied to de-embryonated half barley seeds, they stimulate α–amylase secretion (Taiz and Zeiger, 1998:610). This scientific model has been used for homoeopathic experimentation where Steele (1999) studied the effect of ultra-high dilutions of GA_3 (i.e. 4cH, 9cH, 15cH, 30cH and 200cH) on the α–amylase production in de-embryonated half barley seeds. He demonstrated that ultra-high dilutions of GA_3 are biologically active and noted that there was an observable difference in appearance of the incubated endosperm halves between the control groups and all the treatment groups. These observable differences were due to the fact that starch was not hydrolysed in the control groups whereas it was in the treatment groups. His study compared Hahnemannian and non-Hahnemannian dilutions and utilised de-embryonated half seeds. The control group was treated with extraction buffer containing 58.4 mM sodium chloride and 1.14 mM calcium acetate. Commercial GA_3 only was used to make up the homoeopathic dilutions and not as a separate treatment group.

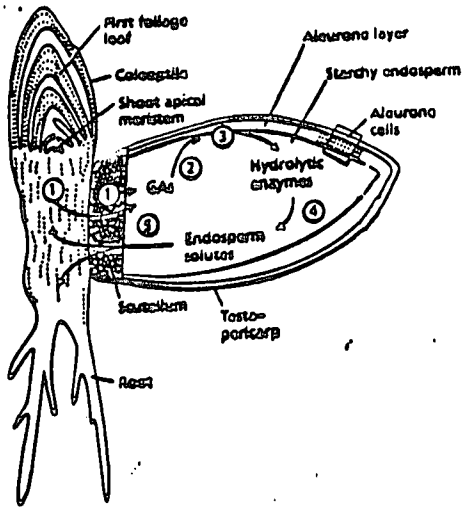

Fig 1.2. The structure of barley seeds and the functions of various tissues during germination. The seed is enclosed in a fused seed coat-fruit wall (testa-pericarp); (1) Gibberellins are synthesised by the coleoptile and scutellum of the embryo and released into the starchy endosperm; (2) gibberellins diffuse to the aleurone layer; (3) the aleurone layer is induced to synthesise and secrete α–amylase and other hydrolases into the starchy endosperm; (4) starch and other macromolecules are broken down to small substrate molecules; (5) the endosperm solutes are absorbed by the scutellum and transported to the growing embryo (indicated by arrows) (Taiz and Zeiger, 1998:609).

Seed Germination

The germination process is the development of a seed into a seedling. The embryo inside a seed is quiescent until it germinates. A number of factors influence whether a seed will germinate successfully. These are environmental factors, including water, oxygen, temperature, and sometimes light requirements (Villee et al, 1985:752). During germination the seed becomes a reproductive unit where the embryo resumes the growth activities which were suspended during quiescence. The seed first takes in water which causes it to expand, breaking the seed coat and initiation of embryo growth follows. To the seed physiologist, germination is defined as the emergence of the radicle through the seed coat. To the seed analyst, germination is "the emergence and development from the seed embryo of those essential structures which indicate the ability of that seed to produce a normal plant under favorable conditions". Presuming that the seed has been in a state of quiescence, or rest, germination is also considered to be the resumption of active growth by the embryo resulting in the rupture of the seed coat and the emergence of a young plant (Copeland and McDonald, 1995:59).

Germination rate is the speed at which a seed is able to germinate over time. Seed lots with similar total germination time often vary in their rate of germination and growth (Copeland and McDonald, 1995:168). Usually the time it takes for 50% of those that did emerge, to emerge, is used as the index for the germination rate of seedlots (i.e. T_{50}). The germination rate of high-vigour seeds is faster than the germination rate of low vigour seeds. The difference between viability and vigour is explained in the case that two batches of seeds are of high viability, since over 80% of the seeds germinated in each

batch. However, in one batch, the seeds are much less vigorous than the other, this being reflected in the T_{50} values where the T_{50} is lower in the less vigorous seeds.

The first sign of germination of barley seed grain in the field is the appearance of the small white coleorihiza (root sheath) at the basal end of the grain. This splits and several seminal roots develop from the coleorihiza. Meanwhile, the coleoptile (leaf sheath) grows and emerges beneath the husk near the apex of the grain. The leaf grows out through a pore at the tip of the coleoptile. Eventually several leaves appear. Guidelines for surveying and distinguishing normal and abnormal seedlings are set out by ISTA (International Seed Testing Association, Appendix A).

Seeds are normally categorised as high medium or low quality seeds. The most convincing and accepted index of seed quality is the ability to germinate therefore germination testing is designed to indicate as closely as possible the proportion of seeds that can be expected to sprout and develop into plants in the field (Copeland and McDonald, 1995:136).

Seed Quality

Seed quality is regarded as an important purchasing criterion by farmers and maltsters due to the fact that high-vigour seeds result in far better germination and seedling development compared to lower-vigour seeds. Vigour tests aid in monitoring seed quality during production and conditioning. As opposed to low vigour seedlots, high-vigour seedlots would be expected to have more vigorous uniform germination and

therefore would be classified as high quality seeds. Medium vigour seedlots will have an average field performance and low vigour seedlots would not perform as well as these. These would be classified accordingly as medium and low quality seedlots. Seed vigour is generally considered as comprising those seed properties that determine the potential for rapid uniform emergence and development of normal seedlings under a wide range of field conditions. This operational definition suggests that vigorous seeds germinate and produce normal seedlings under adverse conditions (Copeland and McDonald, 1989:137). Seeds need to undergo vigour tests and these tests are required to be inexpensive, uncomplicated, rapid, objective, reproducible and related to field performance. Furthermore, vigour is determined under stress conditions (McDonald and Copeland, 1989:137).

The speed of germination is related to seed vigour. Seeds of high vigour germinate rapidly, seeds of low vigour germinate more slowly (Bryant, 1985:53). Other factors that influence the level of seed vigour include the genetic constitution of the seed; environment and nutrition of the mother plant; stage of maturity at harvest; seed size, specific gravity and weight; mechanical integrity; deterioration and aging; and pathogens (Copeland and McDonald, 1989:155).

Regarding the experimental treatment of seeds where germination performance is to be observed, the results from treatment of high quality seed only, should be interpreted with caution because high quality seeds are expected to germinate more readily than medium or low quality seeds. This study therefore investigated the effects of homoeopathically-prepared GA_3 in a range of potencies on germination performance of high-, medium- and

low- quality barley seeds as measured by a germination index. The index involved germination rate and seedling development of the various qualities of barley seed.

Homoeopathic preparations

Dr Hahnemann developed the process of potentisation of his medicines so that he could treat his patients in a gentler manner. As a medical doctor, Hahnemann observed the toxic effects of the high doses of medicines and his intention was to reduce the toxic effects while retaining the therapeutic effects of the medicines by means of the potentisation process (Sankaran, 1991:34). The terms potentisation and dynamisation are synonyms and both refer to the processes of dilution and succussion (Andersch and Endler, 1994:221). The resultant product is referred to as a remedy often prescribed as a medicine for the certain condition it is indicated for (Gaier, 1991:432). This study refers to the resultant product of potentisation as a preparation because the medicinal properties and effects of the prepared substance are not proven in a human and therefore cannot be considered a remedy.

Homoeopathic products are made from mineral compounds, chemicals, poisonous substances and plants (Schofield, 1984). If the original substance is soluble, one part is diluted with nine or ninety-nine parts of distilled water and/or alcohol, and succussed. Succussions involve vigorous shaking of the remedy by raising the container and firmly beating it against the palm of a hand or against a hard surface. This results in the first potency of the remedy i.e. 1D or 1C. Decimal dilutions of 1 to 9 are designated by the Roman numeral x or D (1x = 1/10, 3x = 1/1000, 6x = 1/1,000,000). Centesimal dilutions

of 1 to 99 are designated by the Roman numeral C (1C = 1/100, 3C = 1/1,000,000 etc). In homoeopathy, centesimal dilutions prepared strictly according to the method suggested by Hahnemann are conventionally abbreviated to CH or cH for Hahnemann centesimal. Regarding these abbreviations (cH; CH), the "c" in lower case has been used to refer to "centesimal" in the write-up of this study (i.e. cH). Also, in instances when quoting directly from authors that abbreviated "centesimal" with "C" (uppercase), their form of abbreviation has been used (i.e. CH). "H" (uppercase) refers to "Hahnemannian" in both forms of abbreviations.

Liquid preparations are made according to Method 5a as set out by the German Homoeopharmacopoeia (British Homoeopathic Association, 1985). This method involves solutions produced from basic drug materials and a liquid vehicle. To produce a 1 centesimal dilution (1cH), 1 part of the basic drug material is dissolved in 99 parts of the liquid vehicle and succussed 10 times. The 2nd centesimal dilution is made with 1 part of the 1cH and 99 parts of the liquid vehicle and succussed 10 times according to the German Homoeopharmacopoeia. Subsequent dilutions are produced in the same way. Vehicles listed in the German Homoeopharmacopoeia are absolute ethanol, purified water, glycerol 85%, and ethanol-water mixtures (British Homoeopathic Association, 1985:35-36).

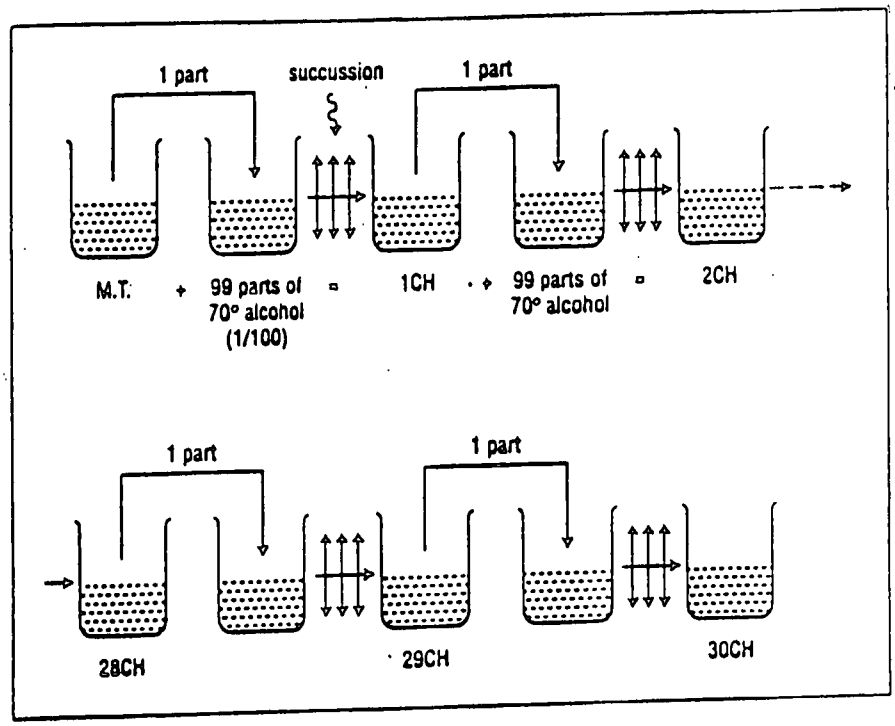

Fig. 1.3 Centesimal Hahnemannian Potentisation Method, using Separate Phials (Gaier, 1991:456).

This study utilised homoeopathic GA_3 in centesimal potencies, therefore dilution levels specific to this scale are given and detail of decimal potency levels is excluded.

Potencies used amongst homoeopaths range from 1cH to 10 000cH or 10M. Table 1.1 (Schofield, 1984) shows the dilution ratio used for making up the respective potency. Included are concentration values of the substance or mother tincture relative to the degree of potency.

Table 1.1 Dilution and Concentration Levels of Centesimal Potencies (Schofield, 1984).

Dilution	Concentration (g L^{-1})	Centesimal potency
$1:10^{2}$	10	1cH
$1:10^{4}$	10^{-1}	2cH
$1:10^{6}$	10^{-3}	3cH
$1:10^{8}$	10^{-5}	4cH
$1:10^{12}$	10^{-9}	6cH
$1:10^{30}$	10^{-27}	15cH
$1:10^{60}$	10^{-57}	30cH
$1:10^{400}$	10^{-397}	200cH
$1:10^{2\,000}$	10^{-1997}	1000cH or M
$1:10^{20\,000}$	10^{-19997}	10 000cH or 10M

Many homoeopaths have found it difficult to explain how homoeopathic medicines act. The ultra-high dilutions challenge scientific understanding (Schulte, 1999). It is impossible to describe the behaviour of something that does not exist (Zacharais and Zacharais, 1997). Many authors have attempted explanations for the action mechanisms of homoeopathic medicines and a main area that is focussed on in homoeopathy is the process of dynamisation (Schulte, 1999; Zacharais and Zacharais, 1997).

Experience has taught us that a substance submitted to dilution and dynamisation is clinically different from a simple dilution (Zacharais and Zacharais, 1997). Dynamisation is technically different due to the periodic dilutions, separated by a number of shakes (succussions). As defined by Zacharais and Zacharais (1997) in their paper on 'Physical Modelling of Dynamisation', the following postulates are defined as follows:

the first postulate establishes that the medicinal properties of an active medicine are transferred in the dynamisation process. It means that this transference can happen even if no material substance is transferred. This effect transforms an inactive medium into an active one and this is based on the fact that no molecule from the mother tincture can be found in higher dynamisation; the second postulate establishes that succussion promotes transference based on the clinical differences between simple dilutions and dynamisations; the third postulate establishes that the quality of the medicinal properties as well as the quantity changes according to the degree of dynamisation, which is based on clinical practice where the dynamisation is changed to obtain better results (Zacharais and Zacharais, 1997).

Consider a preparation of a centesimal dynamisation according to Hahnemann's law. After diluting 1 drop of a cH in 99 drops of inert solvent, a series of succussion results in (n+1) cH. The inert medium can receive the medicinal properties of the active one until it reaches a state of saturation. Even if we continue the succussion, no further properties can be transferred. A new dilution will eliminate the saturation state and the transference continues during subsequent succussion stages until a new saturation state is reached (Zacharais and Zacharais, 1997).

We call the number of molecules per mole Avogadro's number and we know it to be 6.02254×10^{23} mol^{-1}(Majerus, 1991). According to Zacharais and Zacharais (1997) their first postulate lays down that this dynamisation mechanism works even beyond Avogadro's number (approximately 12 cH). The second postulate states that the saturation condition establishes a critical succussion number necessary to saturate an inert

solvent. Beyond this number, further succussions are useless. The third postulate tells us that the efficiency of the transferred properties depends on its degree of dynamisation. This model thus establishes that the dynamisation mechanism requires dilution to eliminate the saturation state and succussion to stimulate the transfer of medicinal properties from one dynamisation to the next. This model is a step towards understanding homoeopathy and use of the dynamisation procedure proposes a model able to stimulate scientific research in order to increase the homoeopathic medicine efficacy (Zacharais and Zacharais, 1997).

The reported actions of highly potentised remedies are the unavoidable consequence of physiochemical changes produced in the course of the potentiation procedure. According to this theory, homoeopathically-potentised preparations have the ability to integrate, contain and improve the original structural information (Resch and Guttman, 1991).

Observability of a system requires that it have the ability to react specifically toward changes in the environment without losing its basic characteristic properties. This ability of maintaining the integrity of the whole system implies that at all times every part knows about every other part in order to respond to changes collectively in the direction to optimum preservation of its form. Intelligence cannot refer to molecules themselves, but rather to the source of their organising ability. Any material system can be divided into different hierarchic levels. These levels remain integrated within the continuity of the whole system and between them, they express differences in dominance (Resch and Guttman, 1991).

Supermolecular System Organisation of Liquid Water

The highest accessible hierarchic level is attributed to the molecules at and near the interface, which provides communication with the environment and exerts the greatest influence on the liquid phase. The interface belongs to liquid water phase and its contacting phase. As long as both phases are maintained, continuous interactions must occur between them and their molecules must be in high states of tension (Resch and Guttman, 1991).

For the existence of liquid water, the presence of gas molecules is required. These are hydrophobic and influence an oscillating pattern of the liquid. These gas molecules have the ability to take over structural information and preserve it dynamically within their oscillating behaviour, in harmony with that of the solution. Subordinate to this level is that of the hydrophylic solutes and their surrounding water molecules, which are always bound to be present in liquid water because of its self-ionization equilibrium with the formation of hydrated ions. The solutes lower the vapor pressure at the interface where a certain contraction is taking place. By addition of hydrophylic solutes to liquid water, the whole solution structure and hence its oscillation pattern is modified. Thus the information from such solutes is spread over the whole solution (Resch and Guttman, 1987:141).

The lowest hierarchic level consists of water molecules relevant for the structural and thermodynamic properties of the liquid and they are subordinate to the solutes and their surrounding hydrophylic water molecules. As external conditions change or more solute

is added to the solution, the hierarchically highest level determines in which ways the whole of the system will respond through the co-operative interactions (Resch and Guttman, 1987:141).

This process is facilitated by shaking the solution since the interface area is tremendously increased during the shaking process, and this leads to more energy being retained in the highest hierarchic level. Existential conflict occurs between the liquid water phase and its contacting phase when shaking the mixture, and energy is redistributed within the developing system organisation. By means of the shaking process, the concentration of dissolved gas molecules is increased. Additional transfer of structural information from the solute molecules to the gas molecules is accomplished because the solute molecules are subordinate to the dissolved gas molecules and the shaking procedure causes the gas molecules to consume a greater amount of energy due to the increase of the interface area (Resch and Guttmann, 1991).

It can therefore be said that the original remedy information is integrated and dynamically maintained in the more dilute solution and that the process of potentiation leads to a substantial improvement of the system organisation of the final (more dilute) solution (Resch and Guttman, 1991).

Mechanisms of Action of Homoeopathic Preparations

The rationale in diluting and potentiating homoeopathic remedies is that the substance which is toxic or suppressive is diluted. By dilution, its noxious properties disappear and

its enhancing properties emerge. The greater the dilution is, the stronger is the enhancing activity of the previous toxic substance (Oberbaum and Cambar, 1994). In order to understand the rationale behind the homoeopathic potentising process, reference to principles and laws that are applicable to homoeopathy and mechanisms of action of homoeopathic preparations are necessary (Schofield, 1984).

The law of similars is usually expressed as "Similia similibus curentur", which means, "Let similars be treated by similars" (Gaier, 1991:264). This law states that any substance which can produce a totality of symptoms in a healthy system can be used to treat a system that naturally developed the equivalent totality of symptoms. This law is applied in clinical practice of homoeopathy where substances that manifested symptoms in a healthy individual are used to treat the similar totality of symptoms in a sick patient (Vithoulkas, 1980:98).

The Arndt-Schultz Law also known as Hueppe's Rule states that substances which inhibit biological processes at sublethal levels may be expected to stimulate them at lower levels. This means that every stimulus on a living cell elicits an activity which is inversely proportional to the intensity of the stimulus. This law is directly related to dose level and the paradoxical responses are produced at low dose levels, which is characteristic of homoeopathy (Scofield, 1984). Concerning the required dosage of drugs, the law of quantity and dose corresponds to the Arndt-Schultz law and is similarly expressed as: The quantity of the drug required is in inverse ratio to the similarity (Gaier, 1991:265).

The 'Law of Initial Value' formulated by Wilder (1957) states that there is a specific inverse relation between the intensity and direction of a response to a stimulus on one hand and the pre-experimental level of a function tested on the other. For example caffeine may prove stimulatory to normal people yet act as a sedative in those already stimulated e.g. restless and agitated children. The initial value of the symptom in the sick organism is obviously at a much higher level than in a healthy organism which does not exhibit the symptom, or exhibits it at a very low level. This law is dependant on the initial value of the function being tested (Schofield, 1984).

The phenomenon of hormesis was proposed by Southam & Elrich (1943) to describe a stimulatory effect of subinhibitory concentrations of any toxic substance on any organism (Schofield, 1984). In various fields of biology, dose-dependant reverse effects of low and very low doses of substances have been used to establish this concept. In many cases, a stimulatory effect occurs in biological systems after exposure to a low concentration of an otherwise toxic agent (Oberbaum and Cambar, 1994). In a review of hormesis, Stebbing (1982) presented numerous examples of growth stimulation in a variety of organisms by substances which are toxic to growth at higher levels. In the many examples given by Stebbing, hormesis only occurs at levels of toxicant which would, by homoeopathic standards, be high and represent very low potencies. He noted that the effect is at the level of the biosynthetic control mechanisms. At high levels the toxicant will inhibit these mechanisms and reduce growth. At low levels the toxicant may exert the same effect but the organism is capable of correcting at the biosynthetic level. It is thought that the biosynthetic mechanisms tend to overcorrect over a limited range of subinhibitory concentrations of toxicants and this results in hormesis (Schofield, 1984).

An example of this dose dependant reverse effect is recognised within conventional medicine. Digitalis is a drug used to manage heart failure in that it increases myocardial contractile force but the drug should be given in low doses and dosage must be carefully monitored. Overdosage leads to digitalis toxicity. Systemic toxic effects include anorexia, vomiting, nausea and life threatening arrhythmias (Berkow et al, 1992).

Examples from agriculture include the use of pesticides where a broad-spectrum pesticide kills many varieties of pests with a single application, and if it is persistent, it does not need to be applied as often. It is said that due to large body size, humans are less susceptible to pesticide compounds such as chlorinated hydrocarbons and anticholinesterases. However, many of these substances are quite toxic, and they sometimes accumulate in soil in amounts that inhibited even plant growth. So, in large doses, serious poisonings especially with potent anticholinesterase pesticides, can occur (Villee et al, 1989:1384). Although hormesis and homoeopathy are neither synonymous nor special cases of each other, it is impossible to overlook the similarities between them especially in those areas that contradict the conventional paradigms. Homoeopathic treatments require symptoms i.e. a system that is depressed or ill, and this is a basic requirement of a hormetic system so as to demonstrate the nullification of some deleterious effects on the environment.

The law of stimuli is formulated as: Minute stimuli encourage life activity, medium to strong stimuli tend to impede it, and very strong stimuli to stop or destroy it. Thus, the actual dose quantities determine the following effects:

i) Stimulation - with small doses a stimulant effect is evoked only.

ii) Inhibition - with moderate doses, the effect is initially stimulating, but becoming depressant later, eventually settling at the starting position.

iii) Toxicity - with large doses, a brief stimulating effect is followed by a depressive sequel which is ultimately lethal (Gaier, 1991:266).

Combining Homoeopathic and Agricultural Sciences

The main goal and principle of homoeopathy as a treatment is to bring about a gentle effect in the individual, through the curative effect of its remedies (Gaier, 1991:265). This is a wholistic approach that encourages the vital force to return to its normal, healthy state and to function in a healthy manner, specific for that life (Sankaran, 1991:35). In the case of illness, a self-healing process comes into effect through this approach (Sankaran, 1991:36). Obviously plant life differs considerably from that of human life, nevertheless the ability of a plant to live or survive relies on the fact that plants have life forces of their own that direct them to grow in a manner specific to the individual plant.

The concept of homoeopathy as a wholistic system of medicine which attempts to raise the level of resistance of an organism to disease or to stimulate its inherent ability to throw off disease is compatible with the wider concepts of biological agriculture (Schofield, 1984). Homoeopathy is generally thought to be a safe method because it is non-polluting both of the environment and of the food product. Also, the medicines are much less expensive than conventional drugs (Schofield, 1984) because minute quantities

of the original substance intended for use are required to make up a homoeopathic preparation of that substance.

With all the pesticides, insecticides, fungicides etc. that are applied to plants in agriculture, much concern is raised as to their toxic effects on plants. The homoeopathic and herbal professions depend upon the availability of pure and unpolluted herbs and plants, because their medicinal qualities will not be expressed unless they are (Cabrera, 1992). If chemical treatments were used to such an extent that they raise concern about toxic effects it would be sensible to research the ability of other available treatments to cause similar effects without the toxic levels that many chemical treatments incur. With the potentisation process used in homoeopathy, toxic levels of chemicals would be of little concern because very little or no molecules of the mother substance would remain in the homoeopathic preparation.

Scientists in agriculture are continuously researching new and advanced ways of enhancing crop development. Similarly scientists in homoeopathy are continuously researching different and more convincing methods to demonstrate the effects of homoeopathic potentisation. Many papers have contributed in both regards, to the development of agro-homoeopathy, many of which are summarised in Schofield's article "Homoeopathy and its Potential Role in Agriculture - A Critical Review" (1984). The field of agro-homoeopathy, offers substantial justification for the use of plant models to demonstrate the efficacy of homoeopathically-prepared substances. Yet, it was found by Schofield (1884) that previous studies that involved plant models did not make any statistical analysis of the data. In some cases no indication at all is given as to why the

experiment was performed. He noted "the failure to identify the rationale of the experiment is a particularly damning criticism of much of the work". Some trials did not indicate the rationale behind the choice of substance that was to be tested. In some trials where a large range of potencies was tested and positive results were obtained these have often been a potencies that are not normally used by homoeopaths. Some of the trials were badly designed and this was evident in some clinical trials where a remedy, rather than the system was tested. In many papers other methodological details, essential for critical assessment and repetition of the work, are missing, e.g. precisely how the controls were organised. In conventional science repeatability of a phenomenon is essential for its general acceptance and this is particularly important in homoeopathy if its validity is to be accepted by the scientific community at large (Schofield, 1984).

Summary

Research that combines homoeopathy and germination performance would contribute to the development of agro-homoeopathy. If designed and controlled carefully, it could be used to investigate the efficacy of homoeopathic preparations and various potencies thereof. This may lead to strong evidence that homoeopathic potentisation is a justifiable method of preparing medicinal substance. Also it has the potential to lead to the development of new methods in which to enhance germination for agricultural use. This may prove beneficial because when preparing a homoeopathic remedy, the original substance intended for use is required in small amounts, thereafter it would undergo potentisation. Thus very little of an expensive chemical would be required during the preparation of a potentially useful growth enhancer.

A distinct advantage of using plant systems is that the effects of homoeopathic preparations in plants cannot be confused with placebo effects i.e. the possibility of suggestion is excluded. Plant models also offer a means with which to demonstrate a direct effect of a homoeopathic substance because plants do not have an immune system in order for them to respond positively. In this way a direct response from a living system is obtainable without ethical implications in the testing of homoeopathic preparations.

It should however, be understood that the effects of homoeopathic preparations demonstrated on plant models cannot be directly transposed to humans and clinical trials. A successful demonstration of the effects of homoeopathic preparations with the use of plant models though, would validate whether or not the principles of homoeopathic potentisation are valid.

To address previous critique of agro-homoeopathy, this study utilised an experimental design that was carefully controlled and easily reproducible and suitable statistical analysis was applied to the data collected.

Objectives

1. To investigate the effect of homoeopathically-prepared dilutions of GA_3 at four levels of potency (4cH, 15cH, 30cH and 200cH) using a plant model i.e. the germination performance of high-, medium- and low- quality whole barley seeds, as measured by a germination index.

2. To assess the potential of various homoeopathic GA_3 potencies to have effects distinct from one another on germination performance of high-, medium- and low- quality barley seeds.

CHAPTER 2

THE EFFECT OF HOMOEOPATHICALLY- PREPARED DILUTIONS OF GIBBERELLIC ACID (GA_3) ON THE GERMINATION RATE OF BARLEY SEED

Introduction

Since homoeopathic remedies in ultra-high dilutions do not contain any active molecule of the original substance by the time of administration, homoeopathy is often disregarded due to claims that the remedies are inactive. However, homoeopathic science claims that throughout the potentisation process, the original remedy information is integrated and dynamically maintained in the more dilute solution (Resch and Guttman, 1992). By demonstrating that homoeopathic preparations are biologically active with the use of plant models, it would support the validity of principles of homoeopathic preparations. In those instances where positive results have been obtained in clinical practice that suggest that homoeopathic preparations are active, these have also been disregarded due to supposition that preparations may have had placebo effect in human subjects. Placebo effect can however be excluded when responses from plant models are obtained.

Homoeopathy is a system of medicine founded by Dr Samuel Hahnemann. He acknowledged that the human body has a tremendous recuperative potential (Sankaran, 1991:36). The body is able to heal through the axis formed by the psyche, nervous, endocrine and immune systems (PNEI-axis). When there are changes in this axis, that is,

when there is an unhealthy mental state associated with disturbed functioning of the nervous, endocrine or immunological systems, naturally these tendencies will result in pathology. The dynamic disturbance which a homoeopathic drug can cause must act through this axis in order to effect a cure for the disturbance and in turn for the pathology (Sankaran, 1991:37). Homoeopathic remedies are prescribed to treat the entire disturbance that has effected this PNEI-axis. Yet, plants do not have a mind, nor do plants have endocrine, nervous, and immune systems in order to obtain a response from the homoeopathic preparation. Plant cells only respond to changes in the environment in which they grow. Exclusion of the possibility of placebo effect and plant immunity would enable observation of a more direct response to homoeopathic treatment, particularly in seed germination. Seeds therefore should provide a suitable test system of the ability of homoeopathic preparations to store and improve the information of a substance in potency (Steele 2000, personal communication).

The germination process of all seeds starts with the adsorption of water. The seed begins to swell and expand which causes the seed coat to break. This process is termed imbibition, during which enzymatic proteins are synthesised and activated and the rate of respiration is increased in the seed. Imbibition ceases after a few hours, and a plateau stage of metabolic activity is reached prior to the emergence of the radicle (Ting, 1982:585). Metabolic activity, involving active RNA synthesis and de novo synthesis of proteins with enzyme activity, is stimulated by plant growth hormones for the mobilisation of food reserves (Ting, 1982:586).

In particular, barley seed (*Hordeum vulgare* L.) germination requires gibberellic action for the mobilisation of endosperm reserves. The coleoptile and scutellum of the embryo synthesise and release gibberellins into the starchy endosperm. Gibberellins then diffuse to the aleurone layer at which synthesis and secretion of α–amylase and other hydrolases occurs in order for the breakdown of starchy endosperm. The endosperm solutes are then absorbed by the scutellum and transported to the embryo for further growth. Cell elongation occurs in two stages. First, a slow elongation of the radicle occurs with only a small increase in fresh weight. This phase may represent active cell wall preparation for the synthesis of new wall materials during later elongation. The second stage is a rapid elongation of the radicle with marked increases in both fresh and dry weight accompanied by rapid mobilisation of nutrients into the radicle (Copeland and McDonald, 1995:90).

An abundance of research on the use of barley half seeds and treatment thereof with GA_3 has established that endogenously applied GA_3 can stimulate α–amylase production and substitute for the embryo (the normal production site of GA) in de-embryonated half seeds (Paleg, 1960; Taiz and Zeiger, 1998:610). Steele (1999) conducted a study in which Hahnemannian and non-Hahnemannian dilutions of GA_3 (4cH, 9cH, 15cH, 30cH and 200cH) were compared. Embryo-less halves of barley seeds were used to investigate the ability of ultra-high dilutions of GA_3 to promote synthesis of α–amylase. Steele (1999) found that starch was not hydrolysed in the control groups whereas it was in the treatment groups and demonstrated that ultra-high dilutions of GA_3 up to 10^{-397} g L^{-1} (i.e. 200cH) are biologically active, with the most active dilution level being 10^{-5} g L^{-1} (i.e. 4cH).

Other plant models have also been used to investigate the efficacy of homoeopathic preparations in seed germination, including studies done on germinability of lettuce seeds (*Lactuca sativa* L.) by Hopkins (1998), and wheat (*Triticum durum*) germination by both Betti et al (1994) and Pongratz & Endler (1994). However, no research with regard to the germination of whole barley seed when treated with homoeopathically-prepared GA_3 can be found.

Germination rate of seeds is the speed at which a seed is able to germinate over time. The index for the rate at which a seed germinates is generally the time it takes for 50% of those that did germinate, to germinate (i.e. T_{50}). Therefore investigation of germination rate in barley seeds in response to homoeopathic treatments would serve as a possible means with which to demonstrate biological activity of homoeopathic preparations. Potentisation is defined as the imparting, along serial dilutions, the pharmacological message of the original substance (i.e. creating a template of the active principle) by means of trituration or succussion (Gaier, 1991:441) and is the process used to produce homoeopathic preparations. By studying the effects of homoeopathically-prepared gibberellic acid (HGA_3) on the germination rate of whole barley seeds, the ability of potentisation to contain and improve the structural information of the original GA_3 substance can be tested.

This study therefore aimed to test the effect of potentisation of GA_3 on the germination rate of barley seeds. Barley seeds of high-, medium- and low-vigour were used. It is understood that seeds of high-vigour are more readily germinable than those of medium-

and low-vigour. In lower quality seedlots some of the seeds may be slow to germinate, ungerminable or dead. Seedlots with similar Total Germination Final Counts often vary in their rate of germination and growth (Copeland and McDonald, 1995:168). Therefore, the inclusion of different vigour seeds is necessary to best investigate the efficacy of HGA_3 on germination rate of whole barley seeds. In addition, the potential of various homoeopathic GA_3 potencies to have effects distinct from one another on germination performance of high-, medium- and low- quality barley seeds can be assessed.

Materials and Methods

All laboratory work was conducted in the Seed Physiology Laboratory, Department of Botany, Agriculture and Natural Sciences, University of Pretoria, Gauteng.

Barley seed was produced in Caledon, Western Cape and obtained from Sensako, Brits. Seed quality was determined (Table 2.1), using vigour tests performed according to the procedures outlined in the rules for testing seeds (International Seed Testing Association, 1995).

Table 2.1 Barley seed quality.

Vigour Level	Cultivar	Standard Germination (%)	Accelerated Ageing (%)
High	SVG 13	98	91
Medium	SVG 13	84	60
Low	SSG 575	86	44

GA_3 was manufactured by Sigma Chemical Co. (St Louis, USA.) and obtained from Separations (Pty) Ltd. Randburg, Gauteng, $C_{19}H_{21}O_6K$ (molecular weight 384.5) containing 90% GA_3 and 10% potassium hydroxide. Potassium hydroxide does not reduce the effect of the GA_3 and has no effect on the synthesis of α–amylase (Steele 2000, personal communication). It is readily soluble in water and was used to prepare homoeopathically-prepared solutions of GA_3 (HGA_3) directly in distilled water. It was preferable to avoid the use of potencies in alcohol, since alcohol may have an inhibitory or stimulatory effect on germination performance. Trituration of GA_3 with lactose up to 3cH (as per standard homoeopathic preparation methods for non-soluble mother

substances - British Homoeopathic Association, 1985:22) was also excluded. Since barley seeds utilise starch among other reserves for germination, it was necessary to eliminate the possibility that lactose might have contributed toward germination performance. GA_3 was supplied to the pharmacist at Natura Homoeopathic Laboratories (Pretoria, Gauteng) in order to prepare HGA_3 3cH, 14cH, 29cH and 199cH, each in 30ml quantities, according to Method 5a as specified in the German Homoeopathic Pharmacopoeia (British Homoeopathic Association, 1985:35-36). HGA_3 potencies of 4cH, 15cH, 30cH and 200cH were each made up in 400ml volumes, using the centesimal potency scale (1:99). In preparing the final potencies, ten succussions were utilised between each potentisation level, but one hundred succussions completed the required potencies. The 15cH, 30cH and 200cH dilutions are above Avogadro's limit so in theory do not contain any molecules of the original GA_3. The 4cH dilution is below this limit.

One hundred barley seeds each of high-, medium-, and low-vigour were allowed to imbibe in 12.5 ml of each of the treatment solutions, at 20°C in the dark (Table 2.2). The cumulative number of germinated seeds was assessed every four hours (seeds were considered germinated once radicle emergence had taken place). There were five replicates. Data were regressed onto a curve as defined by the Gompertz equation (Tipton, 1984), which was used to describe cumulative germination as a function of time. The r^2 of all equations was >98, and the equation from PROC NLIN method=marquardt of SAS was used to calculate T_{50}, i.e., the time it took for 50 percent of those seeds that did germinate, to germinate.

Table 2.2. Imbibition treatment solutions.

Treatment	Dilution	Concentration
Control	distilled water	
GA_3	$5{:}10^6$	$0.5\ g\ L^{-1}$
HGA_3 4cH	$1{:}10^8$	$10^{-5}\ g\ L^{-1}$
HGA_3 15cH	$1{:}10^{30}$	$10^{-27}\ g\ L^{-1}$
HGA_3 30cH	$1{:}10^{60}$	$10^{-57}\ g\ L^{-1}$
HGA_3 200cH	$1{:}10^{400}$	$10^{-397}\ g\ L^{-1}$

Data was analysed as a 6 (incubation medium: control vs GA_3 vs HGA_3 at 4cH vs HGA_3 at 15cH vs HGA_3 at 30cH vs HGA_3 at 200cH) X 3 (seed quality: high vs medium vs lower) factorial treatment classification, in a randomised complete block design (replications = blocks) using PROC GLM of SAS®. Orthogonal contrasts were used to partition treatment effects. It was assumed that errors were normally and independently distributed with mean zero and variance σ^2. There were five replications.

Results

High-Vigour Seeds.

Those seeds imbibed in HGA_3 growth mediums germinated faster than the control group but germination was fastest for seeds imbibed in GA_3 (0.5 g L^{-1}) (Table 2.3). Although biological activity was evident for each potency, there were no significant differences between seeds imbibed in the homoeopathic preparations in terms of germination rate.

Rate of Medium- and Low-Vigour Seeds.

Most of the treatment solutions did not significantly effect germination rate of medium- and low-vigour seeds. Seeds imbibed in HGA_3 growth mediums and GA_3-imbibed seeds did not germinate faster than the control.

Although germination rates for HGA_3 15cH, 30cH and 200cH imbibed- and GA_3 imbibed-seeds of low vigour were not significantly faster than that of the control, it was interesting to note that HGA_3 4cH growth medium had a significantly inhibitory effect on germination rate. Of all the potency levels, the 200cH potency had the least inhibitory effect on germination rate.

Table 2.3. Effect of Gibberellic Acid (GA_3) treatments on whole barley seed germination rate, as measured by time to 50% germination (T_{50}), in hours. Any means followed by the same letters are not significantly different (r=5).

	Vigour		
	High	Medium	Low
Control	23.86a	22.43ab	26.33b
GA_3	20.98c	21.47b	26.08b
HGA_3 4cH	22.31b	22.91a	29.21a
HGA_3 15cH	22.83ab	22.80ab	27.01b
HGA_3 30cH	22.25b	23.58a	26.93b
HGA_3 200cH	22.67b	23.54a	26.67b

Discussion

It was important to include seeds of a range in vigour levels in these studies (Table 2.1), as it is evident that not all vigour levels react in the same way to various growth mediums (Table 2.2). A clearer indication of biological activity was seen in high-vigour seedlots than it would have been if only medium and/or low quality seeds had been utilised for demonstration of biological activity of homoeopathic preparations.

High-vigour seeds imbibed in HGA_3 growth mediums (with the exception of HGA_3 15cH) germinated faster than the control group. Although there were no significant differences between high-vigour seeds imbibed with HGA_3 4cH, 30cH and 200cH in terms of germination rate, it was however demonstrated that these solutions were biologically active. Avogadro's dilution limit is reached in the process of homoeopathic centesimal serial dilution at 12cH (10^{-21} g L^{-1}). In homoeopathic dilutions lower than this, molecules of the original base substance are expected to remain in solution, in homoeopathic dilutions higher than this number, not a single molecule of the original base substance is expected to remain in solution (Gaier, 1991:47-48). The 4cH potency is a dilution lower than Avogadro's number and 30cH and 200cH potencies are dilutions higher than this number. Therefore, potencies both below and above the limit for containing active molecules of GA_3 have biological effects in these germination rate studies.

It has been demonstrated that dilutions of GA_3 at 4cH, and to a far lesser degree, at 9cH, 15cH, 30cH and 200cH, are biologically active since these dilutions stimulated the

synthesis of α-amylase in de-embryonated half-barley seeds (Steele, 1999). However, Steele (1999) did not compare the effects of ultra-high dilutions of GA_3 with commercially applied GA_3, distilled water only was used as the control. Barley seeds of different vigour levels were also not included and de-embryonated halves of barley seeds were utilised. Since barley seeds contain endogenous GA_3, the next step for determining biological effects of homoeopathically potentised GA_3, would be to investigate homoeopathically-prepared GA_3 on the germination of whole barley seeds.

Since whole barley seeds contain their own endogenous GA_3, it was possible that the endogenous GA would have been a limiting factor in which case effects of GA_3 (0.5 g L^{-1}) or HGA_3 for that matter, would not have been seen in germination rate studies. However, high-vigour seeds imbibed in GA_3 (0.5 g L^{-1}) germinated the fastest when compared to other treatment groups, implying that, in spite of the presence of endogenous GA, whole barley seeds were still able to respond to exogenous GA_3. High-vigour seeds were also able to respond to HGA_3 solutions (with the exception of HGA_3 15cH) which demonstrates HGA_3 4cH, 30cH and 200cH the biological activity of high and ultra-high dilutions of GA_3.

Although differences in values between other treatment groups of low-vigour seeds were not significant, a general trend was evident as potency levels increased (i.e. 4cH to 15cH to 30cH to 200cH), germination time decreased (i.e. 29.21 to 27.01 to 26.93 to 26.67 respectively), the fastest of which closely resembled that of GA_3 (i.e. 26.08).

Amongst low-vigour seeds, the homoeopathic potency of GA_3 that was least inhibitory on germination rate was 200cH, that is, the most dilute form of GA_3 and highest potentised solution of the homoeopathic preparations used in the experiment. Although differences were not significant, it was observed that as the level of potency increased, the effect on germination rate tended to become less inhibitory on germination rate.

There is no conclusive explanation for the inhibitory effect of HGA_3 4cH on germination of low vigour seeds, nevertheless, biological activity of the 4cH potency was still clearly demonstrated. In terms of germination rate, HGA_3 4cH imbibition was stimulatory in high-vigour seeds but inhibitory in low-vigour seeds. This suggests that HGA_3 4cH can effect germination rate differently depending on the seed vigour level. This result was of interest because Steele (1999) demonstrated that the 4cH potency of gibberellic acid was highly active since it significantly stimulated the synthesis of α-amylase in de-embryonated halves of barley seeds compared to the effects of other dilutions (i.e. 9cH, 15cH, 30cH and 200cH) used in his study.

The germination rates of medium- and low-vigour seeds however, were unresponsive to imbibition in HGA_3 solutions since they did not germinate significantly faster than their controls. Yet the same was true for GA_3 (0.5 g L^{-1}) imbibed seeds of medium and low vigour levels. Taking into account that seeds were not responsive to GA_3 (0.5 g L^{-1}) treatment together with the fact that endogenous GA was present in all seeds, the biological activity of HGA_3 solutions was not able to be accurately assessed, since it was highly likely that seeds weren't responding to exogenous GA_3 at any concentration/dilution level at medium and low vigour levels. This demonstrates that

lower vigour seeds are less responsive to all these treatment solutions than seeds of high vigour, in terms of germination rate. Therefore, biological effects of HGA_3 in lower-vigour seeds are less likely to be determined, whereas biological effects of HGA_3 4cH, 30cH and 200cH are evident in high-vigour seeds.

Conclusion

In spite of endogenous GA being present in all seeds, whole barley seeds of high-vigour were able to respond to application of exogenous GA_3, where GA_3 (0.5 g L^{-1}) imbibed- and HGA_3 imbibed-seeds (with the exception of HGA_3 15cH), germinated faster than those seeds not receiving GA_3 in any form. Therefore, by increasing the rate at which high-vigour barley seeds germinate, biological activity of homoeopathically-prepared solutions of GA_3 (i.e. HGA_3 4cH, 30cH and 200cH) was clearly demonstrated.

Although high-vigour seeds were able to respond to exogenous GA_3 at different concentration/dilution levels, the same was not evident in medium and low vigour seeds. Therefore, it was concluded that seeds of different vigour levels do not respond in a similar way across all vigour levels due to differing sensitivities to treatment applications.

It cannot be determined why the exception of HGA_3 4cH caused an inhibitory effect in low vigour seeds. This was however a significant effect as it was an example of biphasic action of GA_3 in various concentrations, and clearly demonstrated that HGA_3 4cH is biologically active.

Although it was not possible to determine which potency effected the fastest germination, biological effects of homoeopathically-prepared GA_3 at potency levels of 4cH, 30cH and 200cH were clearly evident. These were however, not significantly large enough for large-scale agricultural/agronomic use.

CHAPTER 3

THE EFFECT OF HOMOEOPATHICALLY- PREPARED DILUTIONS OF GIBBERELLIC ACID (GA_3) ON THE SEEDLING DEVELOPMENT OF BARLEY SEED AS MEASURED BY A GERMINATION INDEX

Introduction

Homoeopathy is often disregarded with claims that homoeopathic remedies do not work since they do not contain any active molecule of the original substance by time of administration (Winston, 1999:451). It is the principle of extremely small doses that generates the greatest controversy surrounding homoeopathy. Sceptics attack this concept, saying that such small doses could not possibly have any effect; homoeopathic medicines are simply placeboes (Ullman, 1992:8). However, homoeopathic science claims that the original remedy information is integrated and dynamically maintained in the more dilute solution (Resch and Guttman, 1992). Homoeopaths and homoeopathic patients have observed for the past two hundred years that the more a substance is potentised (that is, the more it is diluted), the stronger and longer it acts (Ullman, 1992:8). Demonstrating that homoeopathic preparations have an effect on plant systems would contribute to evidence that principles of homoeopathic preparations are valid. By using plant systems, speculations concerning the placebo effect that preparations may or may not have in human subjects, are excluded.

To survive, the human body must continually defend itself against bacteria, viruses, poisons, allergens and both environmental and psychological stresses. The body creates symptoms as its way to adapt to stress or infection as well as to defend and ultimately heal itself (Ullman, 1992:1). Homoeopathic medicine is a natural pharmaceutical science that uses very small doses of substances from the plant, mineral and animal kingdoms to stimulate the body's own defenses (Ullman, 1992:3). Examples of the body's defenses are the immune and nervous systems. When the indicated homoeopathic remedy is able to act upon these systems, health is restored and improvement in the patient's symptoms is observed (Ullman, 1992:11).

As opposed to humans, plants do not have a nervous system and immune system in order to obtain a response from the homoeopathic preparation. By excluding the possibility of placebo effect and plant immunity, a more direct response to homoeopathic treatment is able to be observed and in this study specifically, in seed germination. Seeds therefore can potentially provide a suitable system with which to test the ability of homoeopathic preparations to store and improve the information of a substance.

The germination process of barley seed (*Hordeum vulgare* L.) as with all seeds, involves the uptake of water which causes the seed to swell, and this expansion causes the seed coat to break. After colloidal polymers of the dry seed adsorb water during imbibition, enzymatic proteins are synthesised and activated. Throughout the imbibition stage there is an increase in the rate of respiration. After a few hours imbibition ceases, and the seed reaches a plateau stage of metabolic activity prior to the emergence of the radicle

(Ting, 1982:585). Thereafter metabolic processes directly associated with germination occur. There is active RNA synthesis and de novo synthesis of proteins with enzyme activity. Gibberellin (a plant growth hormone) stimulates the de novo synthesis of α–amylase, proteases and other hydrolytic enzymes that are required for the mobilisation of food reserves (Ting, 1982:586). Gibberellins are synthesised by the coleoptile and scutellum of the embryo and released into the starchy endosperm where the gibberellins diffuse to the aleurone layer. The aleurone layer is then induced to synthesise and secrete α–amylase and other hydrolases so as to breakdown starchy endosperm. The endosperm solutes are then absorbed by the scutellum and transported to the growing embryo.

All gibberellins are acidic and are named GA for gibberellic acid. In particular, GA_3 is highly active and readily available for commercial purposes. Endogenous application of GA to seeds breaks certain types of dormancy and can sometimes accelerate germination of non-dormant seeds (Karssen et al, 1988). In addition to the mobilisation of stored food reserves of the endosperm, seed germination may also require GA_3 for either the activation of vegetative growth of the embryo or the weakening of a growth constraining endosperm layer surrounding the embryo (Dennis & Turpin, 1990:603).

Investigations utilising barley endosperm in de-embryonated half seeds have found that GA_3 can stimulate α–amylase production and substitute for the embryo which is the normal production site of GA (Paleg, 1960; Taiz and Zeiger, 1998:610). There is an abundance of research on the use of barley half seeds and treatment thereof with plant hormones including GA_3. One study in particular (relevant to homoeopathy), compared Hahnemannian and non-Hahnemannian dilutions of GA_3. The ability of ultra-high

dilutions of GA_3 (4cH, 9cH, 15cH, 30cH and 200cH) to promote synthesis of α–amylase was investigated in embryoless halves of barley seeds (Steele, 1999). It was demonstrated that ultra-high dilutions of GA_3 up to 10^{-397} g L^{-1} are biologically active, with the most active dilution level being 10^{-5} g L^{-1} (i.e. 4cH). It was found that starch was not hydrolysed in the control groups whereas it was in the treatment groups (Steele, 1999).

Plant models that focused on seed germination have previously been employed to investigate the efficacy of homoeopathic preparations. These include studies done on germinability of lettuce seeds (*Lactuca sativa* L.) by Hopkins (1998), and wheat (*Triticum durum*) germination by both Betti et al (1994) and Pongratz & Endler (1994). However, no research can be found regarding the seedling development of whole barley seed when treated with homoeopathically-prepared GA_3.

By studying the effects of homoeopathically-prepared gibberellic acid (HGA_3) on the seedling development of whole barley seeds, we can potentially test the ability of potentisation to contain and improve the structural information of the original GA_3 substance. Potentisation also referred to as dynamisation, is defined as the imparting, along serial dilutions, the pharmacological message of the original substance (i.e. creating a template of the active principle) by means of trituration or succussion (Gaier, 1991:441).

Utilising barley seedling development and examining whether the plant expressed natural or unnatural growth in response to homoeopathic treatments should serve as a means with

which to potentially demonstrate biological activity of homoeopathic solutions. By using a range in potencies, the principles that define dose-dependant reverse effects of low and very low doses by homoeopathic standards, will also be able to be investigated. These principles include the biphasic effects of potencies (hormesis), the law of stimuli and the Arndt-Schultz law. These postulates are frequently referred to in homoeopathic literature so as to explain the effects of potencies, dosage and homoeopathic preparations (Schofield, 1984).

It is understood that seeds of high-vigour are more readily germinable than those of medium and low vigour. Therefore, the inclusion of different vigour seeds is necessary to best investigate the efficacy of homoeopathically-prepared GA_3 on germination of whole barley seeds. This study therefore aimed to test the effect of homoeopathic preparations of GA_3 on the seedling development of barley seeds. Whole barley seeds of high-, medium- and low-vigour were therefore treated with HGA_3 4cH, 15cH, 30cH and 200cH.

Materials and Methods

All laboratory work was conducted in the Seed Physiology Laboratory, Department of Botany, Agriculture and Natural Sciences, University of Pretoria, Gauteng.

Barley seed was produced in Caledon, Western Cape and obtained from Sensako, Brits. Seed quality was determined (Table 3.1), using vigour tests performed according to the procedures outlined in the rules for testing seeds (International Seed Testing Association, 1995).

Table 3.1 Barley seed quality.

Vigour Level	Cultivar	Standard Germination (%)	Accelerated Ageing (%)
High	SVG 13	98	91
Medium	SVG 13	84	60
Low	SSG 575	86	44

GA_3 was manufactured by Sigma Chemical Co. St Louis, USA. and obtained from Separations (Pty) Ltd. Randburg, Gauteng, $C_{19}H_{21}O_6K$ (molecular weight 384.5) containing 90% GA_3 and 10% potassium hydroxide. Potassium hydroxide does not reduce the effect of the GA_3 and has no effect on the synthesis of α–amylase (Steele 2000, personal communication). It is readily soluble in water and was used to prepare homoeopathically-prepared solutions of GA_3 (HGA_3) directly in distilled water. It was preferable to avoid the use of potencies in alcohol, since alcohol may have an inhibitory or stimulatory effect on germination performance. Trituration of GA_3 with lactose up to 3cH (as per standard homoeopathic preparation methods for non-soluble mother

substances - British Homoeopathic Association 1985:22) was also excluded. Since barley seeds utilise starch among other reserves for germination, it was necessary to eliminate the possibility that lactose might have contributed toward germination performance. GA_3 was supplied to the pharmacist at Natura Homoeopathic Laboratories (Pretoria, Gauteng) in order to prepare HGA_3 3cH, 14cH, 29cH and 199cH, each in 30ml quantities, according to Method 5a as specified in the German Homoeopathic Pharmacopoeia (British Homoeopathic Association, 1985:35-36). HGA_3 potencies of 4cH, 15cH, 30cH and 200cH were each made up in 400ml volumes, using the centesimal potency scale (1:99). In preparing the final potencies, ten succussions were utilised between each potentisation level, but one hundred succussions completed the required potencies. The 15cH, 30cH and 200cH dilutions are above Avogadro's limit so in theory do not contain any molecules of the original GA_3. The 4cH dilution is below this limit.

One hundred seeds each of high-, medium- and low-vigour were placed in petri dishes (9cm), lined with no. 1 Watmann filter paper, and imbibed with 12.5mL of treatment solution (Table 3.2) for 24 hours in a growth chamber at 20 °C in the dark. After 24 hours, incubated seeds were removed from the petri dishes and transferred by hand onto moist germination towels, enclosed in polyethylene bags and incubated for 6 days in a dark growth chamber at 20 °C.

Table 3.2. Imbibition treatment solutions.

Treatment	Dilution	Concentration
Control	distilled water	
GA_3	$5{:}10^6$	$0.5\ g\ L^{-1}$
HGA_3 4cH	$1{:}10^8$	$10^{-5}\ g\ L^{-1}$
HGA_3 15cH	$1{:}10^{30}$	$10^{-27}\ g\ L^{-1}$
HGA_3 30cH	$1{:}10^{60}$	$10^{-57}\ g\ L^{-1}$
HGA_3 200cH	$1{:}10^{400}$	$10^{-397}\ g\ L^{-1}$

Final germination counts were taken 7 days after start of imbibition, during which the number of normal and abnormal seedlings and dead seeds were assessed. Shoot and root lengths of seedlings were measured. Seedling dry mass was determined using the dry oven method of 60 °C for 30 hours. The above response variables comprised the germination index utilised in this study.

Data was analysed as a 6 (incubation medium: control vs GA_3 vs HGA_3 at 4cH vs HGA_3 at 15cH vs HGA_3 at 30cH vs HGA_3 at 200cH) X 3 (seed quality: high vs medium vs lower) factorial treatment classification, in a randomised complete block design (replications = blocks) using PROC GLM of SAS®. Orthogonal contrasts were used to partition treatment effects. It was assumed that errors were normally and independently distributed with mean zero and variance σ^2. There were three replications.

Data from an initial replicate was excluded from analysis (Appendix B). This served as a technique-refinement run which helped to improve the protocol for the subsequent three replicates.

Results

Shoot Length

Those seeds imbibed in GA_3 resulted in seedlings with longer shoots, and this was evident across all vigour levels (Fig. 3.1). The difference in shoot length between those seedlings and those of the control group (seeds to which no treatment had been applied) was, however, only significant in the low-vigour seed lot.

Imbibing seeds in homoeopathically-prepared GA_3 (HGA_3) had no effect on shoot growth, regardless of the seed vigour level or potency level.

Root Length

Across high- and medium-vigour levels, those seeds imbibed in GA_3 growth mediums did not produce roots that were significantly shorter than those of seeds of the control. However, significant inhibitory effects on roots were seen when GA_3 (0.5 g L^{-1}) was used for imbibing low-vigour seeds (Fig 3.2). This implied that GA_3 had a significantly inhibitory effect on seedling root development in low-vigour seeds, whereas it had no effect on seeds of high- and medium-vigour.

Statistical differences were not seen when comparing root lengths of HGA_3 4cH, 30cH and 200cH solutions to those of the control. Of medium-vigour seeds, only HGA_3 15cH stimulated root development more successfully than both GA_3 (0.5 g L^{-1}) and the control. This was particular evidence of biological activity in homoeopathic preparations at this ultra-high dilution level (i.e. 10^{-27} g L^{-1}). In this case, biphasic effects of GA_3 were demonstrated. The biphasic effect was seen when the direct effect on root growth was

inhibitory in GA_3 (0.5 g L^{-1}) imbibed seeds and the opposite effect was observed with HGA_3 15cH imbibed seeds in which case, root development was stimulated.

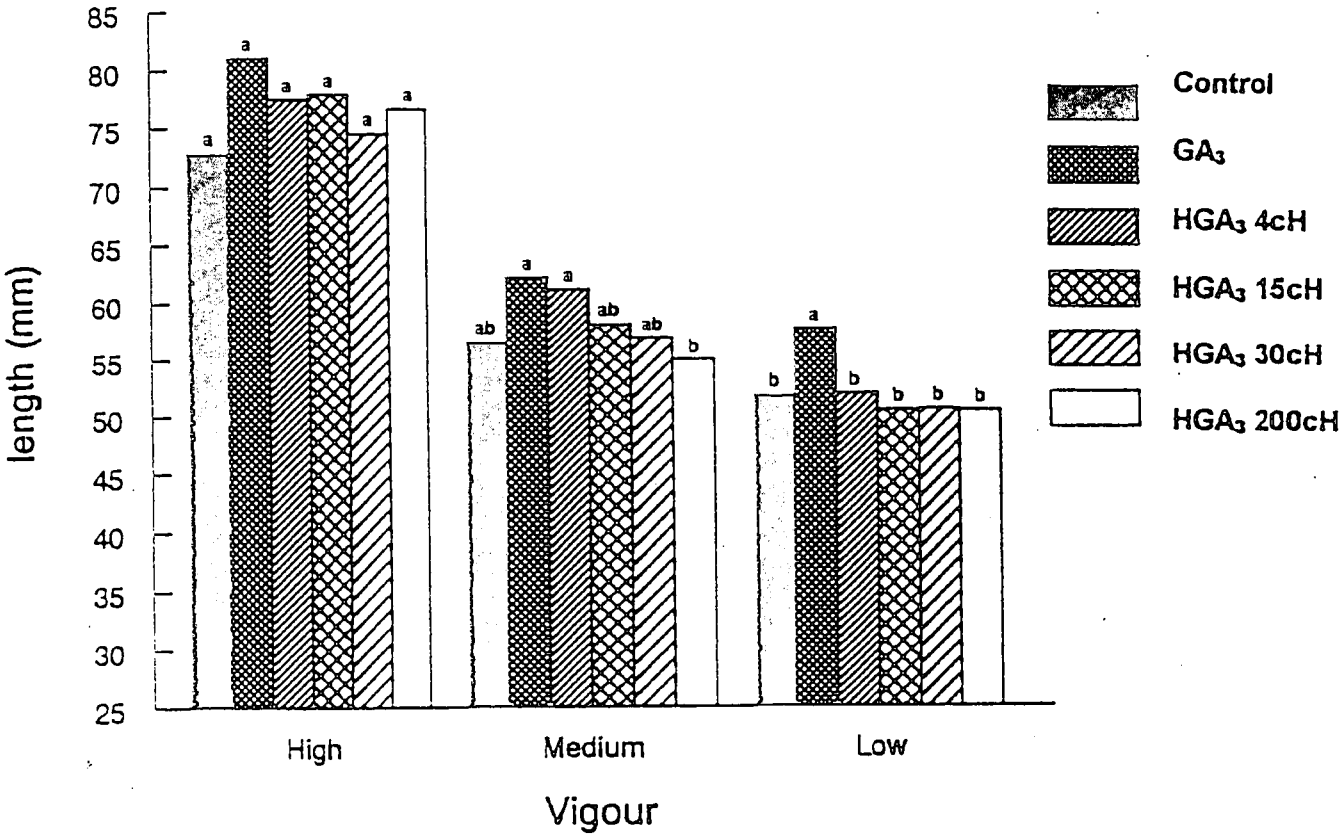

Fig 3.1. The effect of imbibing whole barley seeds ranging from high to low vigour in GA_3 (0.5 g L^{-1}) and homoeopathically prepared GA_3 (HGA_3 4cH, 15cH, 30cH and 200cH) on subsequent shoot length. Any means bars with the same letters above are not significantly different.

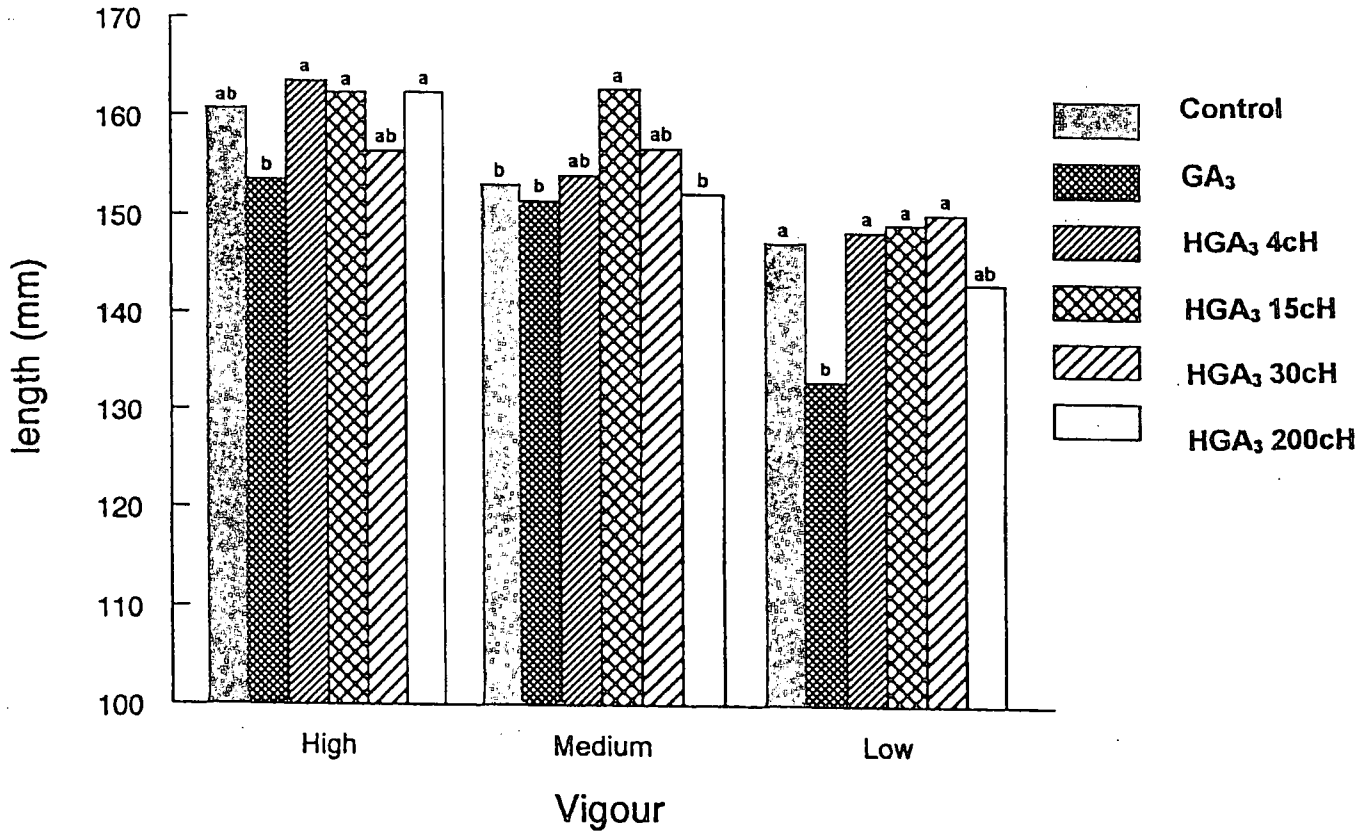

Fig 3.2. The effect of imbibing whole barley seeds ranging from high to low vigour in GA_3 (0.5 g L^{-1}) and homoeopathically prepared GA_3 (HGA_3 4cH, 15cH, 30cH and 200cH) on subsequent root length. Any means not followed by the same letters are not significantly different.

Seedling Mass

Inhibition of total seedling mass across all vigour levels was significant in seeds that were imbibed in GA_3 (0.5 g L^{-1}). Compared to the control, inhibitory effects of GA_3 were significant only in high-vigour seeds, but when compared to HGA_3 solutions, GA_3 was significantly inhibitory across all vigour levels in terms of total seedling mass.

High-vigour seeds imbibed in HGA_3 solutions developed seedlings that were larger than those of the control and those of GA_3 imbibed seeds. The most highly potentised solution i.e. HGA_3 200cH, produced the biggest seedlings. Following this, bigger to smaller seedlings were found in the order of HGA_3 15cH, HGA_3 4cH and HGA_3 30cH imbibed seeds. Compared to these, seedlings that developed from seeds imbibed in GA_3; and seedlings of the control group, were significantly smaller in size. All HGA_3 solutions therefore had a stimulatory effect on seedling growth of high-vigour seeds, indicating biological activity in the 4cH, 15cH, 30cH and 200cH potencies.

Seedling mass was significantly higher for all HGA_3 imbibed seeds (with the exception of HGA_3 30cH) in medium-vigour seeds compared to seedling mass of the control and of GA_3 imbibed seeds. It was also evident that HGA_3 30cH imbibed seeds were more successful in producing larger seedlings than GA_3 imbibed seeds.

Of medium-vigour seeds, the biggest seedlings were developed from HGA_3 200cH imbibition, which were significantly larger than seedlings of HGA_3 4cH imbibed-, 30cH imbibed- and GA_3 imbibed seeds, and the control. Seedling mass of HGA_3 15cH

imbibed seeds did not differ from that of 4cH and 200cH imbibed seeds. However all these seedlings were comparatively larger than those of the control and GA_3 imbibed seeds. This implies that for medium-vigour seeds, HGA_3 4cH, 15cH and 200cH solutions had a stimulatory effect on seedling growth which thus supports previous evidence of biological activity in the 4cH, 15cH, and 200cH potencies.

Seedling mass was also significantly higher for all HGA_3 imbibed seeds (with the exception of HGA_3 30cH) in low-vigour seeds compared to seedling mass of the control and of GA_3 (0.5 g L^{-1}) imbibed seeds. There were no significant differences amongst the HGA_3 imbibed low-vigour seeds, however, it was clear that for low vigour seeds, HGA_3 4cH, 15cH and 200cH solutions had a stimulatory effect on seedling growth and again substantiates previous evidence of biological activity in the 4cH, 15cH, and 200cH potencies.

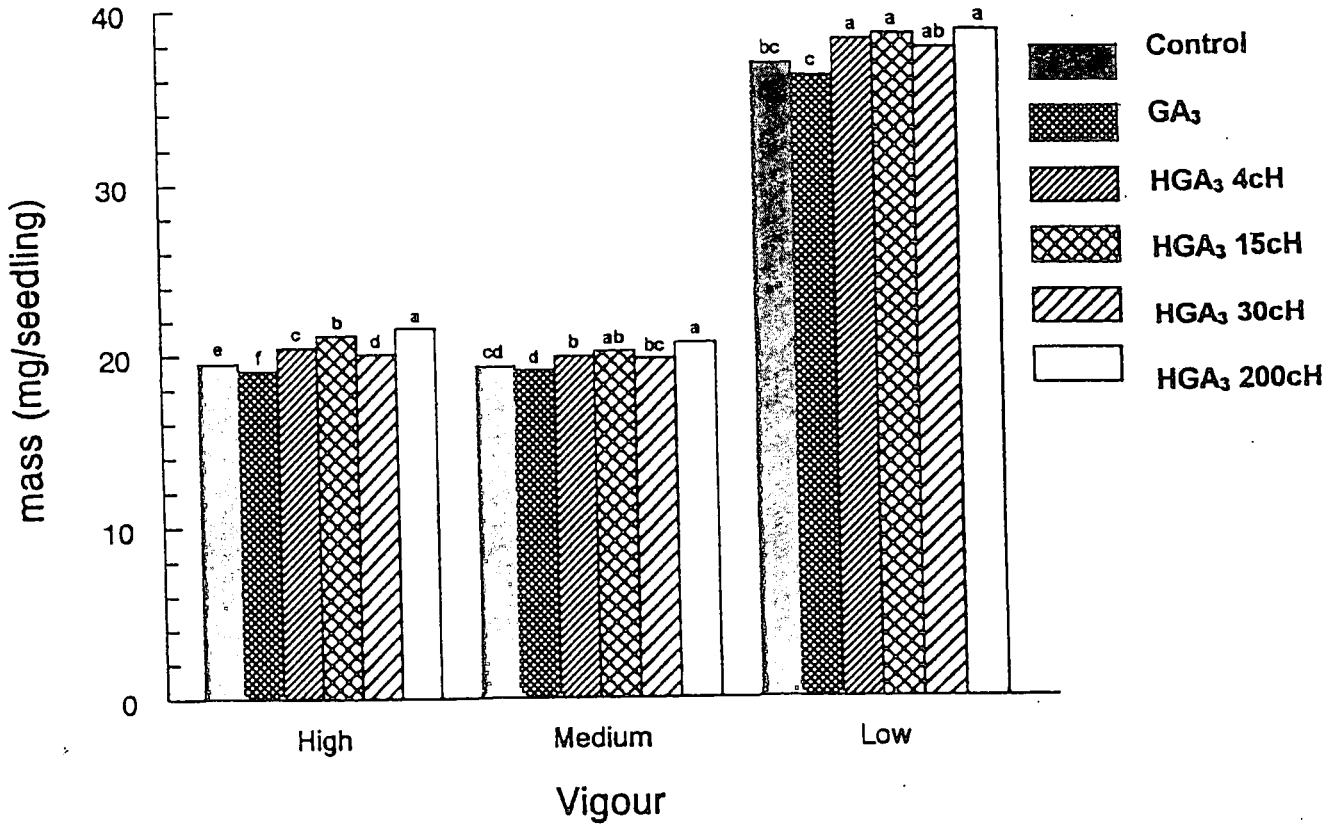

Fig 3.3. The effect of imbibing whole barley seeds ranging from high to low vigour in GA_3 (0.5 g L^{-1}) and homoeopathically prepared GA_3 (HGA_3 4cH, 15cH, 30cH and 200cH) on subsequent seedling mass. Any means not followed by the same letters are not significantly different.

Normal Seedling Development.

Final germination counts for high-, medium-, and low-vigour seedlots, after seven days are listed in Tables 3.3 – 3.5. Vigour groups were analysed statistically separately from one another. No statistically significant differences were observed with regard to the number of normal, abnormal seedlings and dead seeds when treated with distilled water, GA_3 (0.5 g L^{-1}), HGA_3 4cH, 15cH, 30cH and 200cH solutions.

Table 3.3 Normal seedlings of high-, medium-, and low-vigour seedlots, at final count after 7 days.

	Vigour		
	High	Medium	Low
	----------%----------		
Control	87.0	77.0	75.0
GA_3	84.7	77.7	74.3
HGA_3 4cH	82.7	84.7	71.3
HGA_3 15cH	93.0	73.0	76.3
HGA_3 30cH	89.0	82.3	76.3
HGA_3 200cH	90.7	79.7	72.0

Table 3.4 Abnormal seedlings of high-, medium-, and low-vigour seedlots, at final count after 7 days.

	Vigour		
	High	Medium	Low
	----------%----------		
Control	9.0	11.0	20.0
GA_3	12.3	11.0	20.3
HGA_3 4cH	16.0	6.0	23.0
HGA_3 15cH	5.7	11.0	17.0
HGA_3 30cH	10.0	9.0	20.0
HGA_3 200cH	6.7	11.0	22.3

Table 3.5 Dead seeds, of high-, medium-, and low-vigour seedlots, at final count after 7 days.

	Vigour		
	High	Medium	Low
	----------%----------		
Control	4.0	11.3	5.0
GA_3	3.0	11.3	5.3
HGA_3 4cH	1.3	9.3	5.7
HGA_3 15cH	1.3	16.0	6.7
HGA_3 30cH	1.0	8.7	3.7
HGA_3 200cH	2.7	9.3	5.7

Discussion

Seedling development of seeds imbibed in a GA_3 (0.5 g L^{-1}) solution demonstrated biological effects of exogenous GA_3. Under normal circumstances, endogenous GA_3 is present in whole barley seeds. Thus, germination performance in this study of whole seeds may not have responded to the extra (exogenous) GA_3 since the seeds already contained sufficient endogenous GA_3. Had GA_3 0.5 g L^{-1} (a more concentrated solution of GA_3) not shown biological effects on seedling development, it would not be likely for homoeopathically-prepared GA_3 solutions (i.e. ultra-high dilutions) to effect germination performance.

The effects of imbibing seeds in GA_3 (0.5 g L^{-1}) with regard to seedling development included increased shoot growth, and the inhibition of root growth observed in low-vigour seeds. Additionally, GA_3-imbibed seeds of high vigour developed small seedlings compared to those of the control and HGA_3 imbibed seeds.

Biological activity is evident in homoeopathically-prepared GA_3 (HGA_3) 4cH, 15cH, 30cH and 200cH potencies. Seedlings treated with individual HGA_3 solutions were significantly larger compared to those of the control and GA_3 (0.5 g L^{-1}) treatment. The fact that all HGA_3 solutions stimulated seedling growth when high-vigour seeds were used, clearly indicated biological activity in the 4cH, 15cH, 30cH and 200cH potencies.

In previous studies that utilised half-barley seeds, the use of GA_3 has often been at concentrations below Avogadro's number (i.e. potencies below 12cH or concentrations stronger than 10^{-21} g L^{-1}).

Van der Meulen et al (2000), studied the effects of fusicoccin and gibberellic acid on the germination of embryos from dormant barley grains. It was demonstrated that 10^{-8} g L^{-1} and 10^{-6} g L^{-1} GA_3 were able to induce about 40% and 70% germination, respectively. In embryos isolated from dormant grains GA_3 (10^{-6} g L^{-1}) was able to elicit maximum induction of α-amylase mRNA expression. Similar to the HGA_3 4cH potency, 10^{-8} g L^{-1} and 10^{-6} g L^{-1} GA_3 are below Avogadro's number (12cH i.e. 10^{-21} g L^{-1}) and it is noted that these concentrations are more dilute than HGA_3 4cH (i.e. 10^{-5} g L^{-1}). The biological activity of GA_3 (10^{-8} g L^{-1} and 10^{-6} g L^{-1}) was thus observed, yet this was possible in a plant model that utilised isolated embryos from dormant barley grains.

Therefore, it has been demonstrated that dilutions of GA_3 above 10^{-5} g L^{-1} (i.e. 10^{-8} g L^{-1} and 10^{-6} g L^{-1}) are biologically active (Van der Meulen et al, 2000). These remain to be dilutions of GA_3 that are below Avogadro's number and the method of dilution was not that of serial dilution nor was succussion included in the preparation of the GA_3 that was utilised in the study by Van der Meulen, et al. (2000).

A previous study has demonstrated that ultra-high dilutions of GA_3 (4cH, 9cH, 15cH, 30cH and 200cH) are biologically active since these dilutions stimulated the synthesis of α-amylase in de-embryonated half-barley seeds (Steele, 1999). Therefore, the investigation of homoeopathically potentised GA_3 on the germination of whole barley

seeds was an interesting way in which to investigate biological effects of homoeopathically potentised GA_3 in the presence of endogenous GA_3 which is under normal circumstances, found in barley seeds.

The investigation of HGA_3 on the seedling development of whole barley seeds, demonstrated that HGA_3 200cH was significantly more effective than HGA_3 4cH in the development of larger seedlings in high- and medium-vigour seedlots (Fig 3.3). Interestingly, Steele (1999) demonstrated that a 4cH potency of GA_3 was considerably more effective than the 200cH concentration in the synthesis of α-amylase in de-embryonated barley seeds.

The dramatic result of the 4cH potency that was obtained by Steele (1999) in comparison to other homoeopathic potencies that were tested, is possibly due to the fact that it was a more concentrated solution of GA_3 (i.e. 10^{-5} g L^{-1}). It has been demonstrated that even smaller concentrations of GA_3 are required to obtain α-amylase synthesis in de-embryonated barley seeds. Van der Meulen et al, (2000) utilised GA_3 in dilutions of GA_3 (10^{-8} g L^{-1} and 10^{-6} g L^{-1}) that were slightly higher than the 4cH (i.e. 10^{-5} g L^{-1}).

Although previous studies that utilised de-embryonated barley seeds demonstrated that dilutions of GA_3 which are lower than Avogadro's limit have biological effect, we would assume that these solutions are more active because they contain a higher concentration of GA_3 molecules than homoeopathically-prepared GA_3 15cH, 30cH and 200cH, since these homoeopathic potencies are ultra-high dilutions of GA_3. Theoretically, no molecules of the original substance should remain in solution when dilution has exceeded

Avogadro's limit. Since HGA_3 15cH, 30cH and 200cH no longer contain traces of GA_3, sceptics of homoeopathic preparations would therefore not expect HGA_3 in these potencies to effect seedling development any different to that of the application of distilled water (control) (Winston, 1999:450). However, compared to seedlings that developed from seeds imbibed in GA_3; and seedlings of the control group, all HGA_3 solutions had a stimulatory effect on seedling growth of high-vigour seeds, thus indicating biological activity in the 4cH, 15cH, 30cH and 200cH potencies.

Since HGA_3 4cH is a dilution below Avogardo's limit, and HGA_3 15cH, 30cH and 200cH are ultra-high dilutions above this limit, it can be concluded that all these potencies, which represent potencies that are normally used by homoeopaths (Jouanny, 1993:83; Vithoulkas, 1990:217), exhibit biological activity.

These results challenge assumptions that homoeopathic remedies are inactive since not a single molecule of the original substance exists in solutions that have been potentised beyond Avogadro's limit (Winston, 1999:451).

Hormesis has been defined as 'in many cases a stimulatory effect occurs in biological systems after exposure to a low concentration of an otherwise toxic agent' (Oberbaum and Cambar, 1994). This concept was used to interpret the biphasic drug action set out by Hahnemann as 'Most medicines have a first direct effect that changes gradually into the second effect that is opposite to the first' (Gaier, 1991:185). The hormesis phenomenon however does not encompass ultra-high homoeopathic dilutions according to Schofield (1984). According to Oberbaum and Cambar (1994), hormesis is not

homoeopathy because the "classic" hormetic systems act at concentrations that are much higher than those used in homoeopathy. Yet biphasic action of GA_3 was demonstrated in the effects of: HGA_3 4cH, 15cH and 200cH on the root development of high-vigour seeds and HGA_3 4cH, 15cH and 30cH on the root development of low vigour seeds. The effects that were demonstrated in seedling root growth by ultra-high dilutions of HGA_3 15cH, 30cH and 200cH, therefore negate the suggestions made by Schofield (1984), Oberbaum and Cambar (1994).

A concentration of GA_3 0.5 g L^{-1} was used, a relatively small dilution when compared to HGA_3 solutions, some of which are ultra-high dilutions. It was evident that homoeopathic preparations are biologically active in ultra-high dilutions of 10^{-27} g L^{-1} (i.e. 15cH) since HGA_3 15cH stimulated root development in medium-vigour seedlings more successfully than both GA_3 and the control. In contrast to this, GA_3-imbibed seeds of medium vigour showed inhibitory root development. This was evidence of biological activity in homoeopathic preparations at an ultra-high dilution level (i.e. 10^{-27} g L^{-1}). In this case, biphasic effects of GA_3 were also demonstrated.

The law of stimuli is applicable to mechanisms of homoeopathic potency action. This law, which is formulated as "Minute stimuli encourage life activity, medium to strong stimuli tend to impede it, and very strong stimuli to stop or destroy it" (Schofield, 1984), is demonstrated in medium vigour seedlings since GA_3 (0.5 g L^{-1}) (the considerably stronger stimulus) caused inhibition of root growth, whereas the HGA_3 15cH ultra-high dilution 10^{-27} g L^{-1} (the smaller stimulus) stimulated root development. The law of stimuli correlates well with the Arndt-Schultz law which states that substances inhibiting

biological processes at sublethal levels may be expected to stimulate them at lower levels (i.e. more diluted levels).

Schofield (1984) states that the Arndt-Schultz law may be a useful scientific explanation of homoeopathic activity but can only be true at low potencies e.g. 4cH. However, it was found that homoeopathic dilutions as high as 10^{-27} g L^{-1} (HGA_3 15cH) (in medium-vigour seed) responded according to the Arndt-Schultz law, and the law of stimuli in the sense that HGA_3 15cH solution stimulated root development which was opposite to the decreased root growth in GA_3 0.5 g L^{-1} treated seeds.

In instances where stimulatory effects on some HGA_3-imbibed seeds directly contrasted with inhibitory effects seen in GA_3-imbibed seeds, it can be concluded that important properties which are not evident in a drug's crude state, are only manifested in potency. This was demonstrated in seedling development across all vigour levels, with the use of HGA_3 4cH, 15cH, 30cH and 200cH potencies in their stimulatory effect on seedling mass, whereas GA_3-imbibed seeds were smaller across all vigour levels. In addition, amongst medium-vigour seedlings, the HGA_3 15cH potency had a stimulatory effect on root development whereas root development was inhibited in GA_3-imbibed seeds.

Since dry mass was significantly increased by imbibing seeds in HGA_3 solutions, cell production and elongation was probably increased in those seeds. This phenomenon supports the concept of homoeopathy as a wholistic treatment, where the entire system is stimulated towards a more uniform and balanced growth. Of all the HGA_3 solutions,

HGA_3 200cH was the most highly diluted or rather most highly potentised solution, and was responsible for producing the largest seedlings across all vigour levels.

Biological activity was evident in all HGA_3 solutions yet it was not possible to speculate as to which particular HGA_3 potency was most beneficial with regard to seedling mass development, if indeed there were differences amongst potency levels. Although HGA_3 200cH stimulated high and medium vigour seeds to grow larger than other treated seeds, seedling sizes of HGA_3 4cH, 30cH and 200cH treatments did not differ to each other in low vigour seeds.

Since whole barley seeds were utilised, it must be kept in mind that barley seed embryos have sufficient GA_3 for germination requirements. The failure of HGA_3 to demonstrate a biological effect in some cases is not evidence that homoeopathy itself has failed, instead it was highly probable that this may have been due to sufficient quantities of endogenous GA_3 in the barley seeds.

If it were true that homoeopathic preparations are not biologically active, there should have been no differences in terms of shoot or root development, or seedling mass, between the control and the HGA_3 solutions. Yet many significant differences were observed between the control and HGA_3 4cH, 15cH, 30cH and 200cH solutions. There were numerous instances where HGA_3 solutions were responsible for increased seedling sizes in seeds across all vigour levels. This clearly demonstrated biological activity of HGA_3 4cH, 15cH, 30cH and 200cH solutions.

This is strong evidence that ultra-high homoeopathic dilutions of an original substance remain biologically active and that it is unjustified to consider homoeopathic preparations as effective as plain water or alcohol.

Conclusion

Potentisation is a physical process through which latent properties of crude substances are aroused into activity, though these may have not been evident in their crude states (Gaier, 1991:444). This has been successfully demonstrated in the seedling development of barley seeds where various mechanisms of homoeopathically-potentised gibberellic acid (HGA_3 4cH, 15cH, 30cH and 200cH) were observed in the various aspects of the seedling development of barley.

Biphasic action of GA_3 in various dilutions was observed in medium-vigour seeds where GA_3 inhibited root development, whereas the HGA_3 15cH potency stimulated root development. Therefore the homoeopathic ultra-high dilution of 10^{-27} g L^{-1} demonstrates biological activity of the 15cH potency and supports Hahnemann's description of biphasic action.

Biological effects of HGA_3 potencies (4cH, 15cH, 30cH and 200cH) were observed amongst high-vigour seeds, where all the HGA_3-imbibed seeds produced significantly larger seedlings than those of GA_3 (0.5 g L^{-1}) imbibed seeds. This therefore demonstrates the biological activity of HGA_3 potencies below and above Avogadro's limit for any remaining molecules of the original base substance (i.e. 4cH - below this limit, 15cH, 30cH and 200cH - above this limit).

The biological effects of HGA_3 15cH was demonstrated in medium-vigour root development; and of HGA_3 4cH, 15cH, 30cH and 200cH were demonstrated in the effect

on high-vigour seedling mass. In these instances, biphasic effects of GA_3 in various concentrations, were demonstrated. The considerably stronger stimulus of GA_3, was inhibitory in terms of seedling size compared to high and ultra-high homoeopathic dilutions of GA_3. All HGA_3 imbibed seeds resulted in bigger seedlings than those of GA_3 imbibed seeds. Biphasic effects were demonstrated amongst medium vigour seeds, HGA_3 15cH stimulated root development compared to GA_3 0.5 g L^{-1} which inhibited root development. In these effects, the mechanisms of potency action explained according to the laws of stimuli and Arndt-Schultz were substantiated where GA_3 activity is stimulating in weak doses (i.e. homoeopathic potencies) and is inhibitory in stronger doses (i.e. GA_3 0.5 g L^{-1}).

Homoeopathically-prepared solutions of GA_3 have the potential to elicit a biological response, even though they were incredibly highly diluted. Of the homoeopathically-prepared GA_3 solutions, 200cH was the most highly diluted, or most highly potentised solution, and yet imbibition of high-, medium- and low-vigour seeds with HGA_3 200cH resulted in the largest seedlings compared to those of other treatment groups. Therefore it was demonstrated yet again, that homoeopathic potencies are biologically active in ultra-high homoeopathic dilutions as high as 10^{-397} g L^{-1} (i.e. 200cH).

Clearly then, although homoeopathic preparations contain none, or very few molecules of the original substance (dependant on the potentisation level), GA_3 in high- and ultra-high homoeopathic dilutions exhibited biological activity.

CHAPTER 4

FINAL CONCLUDING REMARKS

In both germination rate and seedling development experiments it was demonstrated that HGA_3 4cH, 15cH, 30cH and 200cH are indeed biologically active in the presence of endogenous GA_3. It can also be concluded that seeds of different vigour levels do not respond similarly across all vigour levels presumably due to differing sensitivities to treatment applications. Each potency (4cH, 15cH, 30cH and 200cH) expressed distinct biological effects in studies of germination rate and seedling development. Biological effects of individual potencies were exhibited for example, in the instance that homoeopathically-prepared GA_3 at 15cH stimulated root development in medium-vigour seeds; homoeopathically-prepared GA_3 at 4cH inhibited rate of germination in low-vigour seeds; high-vigour seeds imbibed in homoeopathically-prepared GA_3 at 30cH resulted in larger seedlings; and homoeopathically prepared GA_3 200cH stimulated the greatest seedling development across all vigour levels. Also, by increasing the rate at which high-vigour barley seeds germinate, biological activity of homoeopathically-prepared solutions of GA_3 (i.e. HGA_3 4cH, 30cH and 200cH) was clearly demonstrated.

Although homoeopathically-prepared GA_3 stimulated barley seed germination in terms of seedling sizes in high-vigour seeds and homoeopathically-prepared GA_3 at 15cH stimulated root development in medium-vigour seeds, these were however, not significantly large enough for large scale agricultural/agronomic use.

By applying individual homoeopathically-prepared GA_3 solutions to barley seeds, some effects not evident of GA_3 in its crude state, are indeed manifested in potency. The potentisation process is able to integrate and dynamically maintain structural information of the original GA_3 since homoeopathically-prepared GA_3 was able to stimulate germination rate (in high-vigour seeds) and larger seedling growth (across all seed vigour levels) despite endogenous GA_3 in the seed.

Homoeopathic principles and precepts such as Hahnemann's description of biphasic effects (hormesis), the law of stimuli and the Arndt-Schultz law were supported by biphasic effects that were found in these studies. It can be concluded that biphasic effects are attainable when using homoeopathic ultra-high dilutions such as 10^{-27} g L^{-1}, 10^{-57} g L^{-1} and 10^{-397} g L^{-1} (i.e. 15cH, 30cH and 200cH respectively).

Individual ultra-high dilution levels of 15cH, 30cH and 200cH potencies are attenuated during the potentisation process, to above Avogadro's limit and therefore do not contain any molecules of the original substance from which they were prepared. Yet it was evident from germination rate and seedling development studies that homoeopathically-potentised substances are biologically active in barley seed germination.

RECOMMENDATIONS

It is recommended that:

1. Future plant model experiments examining germination should include seeds of various vigour levels, when testing the efficacy of homoeopathic preparations.
2. Seeds other than barley, at various vigour levels be utilised to test the efficacy of homoeopathically-prepared gibberellic acid in potencies normally used by homoeopaths.
3. Homoeopathically-prepared gibberellic acid in potencies 4cH-200cH be used to moisten germination towels before incubation of imbibed seeds as opposed to only applying homoeopathically-prepared GA_3 at imbibition.
4. The same experimental model (the effect of homoeopathically-prepared dilutions of growth hormones on the germination of barley seed as measured by a germination index) be used to test other plant hormones in various potencies normally used by homoeopaths.

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LIST OF APPENDIXES

APPENDIX A

Normal seedling growth will be surveyed in terms of the following:

a) **root system:** at least two seminal roots intact or with only slight defects:

e.g. - discoloured or necrotic spots.

b) **shoot system:**

the mesocotyl, intact or with only slight defects

e.g. - discoloured or necrotic spots

the coleoptile, intact or with only slight defects

e.g. - discoloured or necrotic spots

- loose twists

- split from one third or less from the tip

the leaf intact, emerging through the coleoptile near the tip or with only slight defects

e.g. - discoloured or necrotic spots

- slightly damaged

c) **seedling:** all the essential structures normal as detailed above

4.2.2 Abnormal seedling growth will be surveyed in terms of the following:

a) **root system:** the seminal roots defective or insufficient

e.g. - stunted or stubby

- retarded

- only one or completely missing

- broken

- constricted

- spindly

- glassy

- decayed as a result of primary infection

- with negative geotropism

b) **shoot system:**

the mesocotyl, (where developed) defective

e.g. - broken

- decayed as a result of primary infection

the coleoptile, defective

e.g. - deformed (e.g. short and thick due to phyto-toxic effect)

- broken

- missing

- with the tip damaged or missing

- forming a loop or spiral

- tightly twisted

- strongly bent over

- split for more than one third of length from the tip

- split at the base

- spindly

- decayed as a result of primary infection

the leaf, defective:

e.g. - extending less than half-way up the coleoptile

- missing

- shredded or otherwise deformed

c) **seedling:** one or more of the essential structures abnormal as detailed above, or normal development prevented, because the seedling as a whole is defective

e.g. - deformed

- two fused together

- yellow or white

- spindly

- glassy

- decayed as a result of primary infection.

(International Seed Testing Association, 1995).

Rep 1

	SHOOT LENGTH					ROOT LENGTH				
	1	2	3	4	Average	1	2	3	4	Average
CONTROL HIGH	90.30	87.80	106.40	87.30	92.95	143.30	132.30	147.50	131.50	138.65
CONTROL MEDIUM	72.00	74.60	69.90	71.80	72.08	137.40	132.60	123.80	120.10	128.48
CONTROL LOW	70.20	62.80	55.50	67.00	63.88	141.90	124.20	125.00	126.20	129.33
GA_3 HIGH	99.10	92.10	98.40	89.19	94.70	148.20	132.42	122.06	131.86	133.64
GA_3 MEDIUM	84.04	80.19	78.64	95.46	84.58	130.30	125.80	125.66	140.26	130.51
GA_3 LOW	99.67	97.67	91.15	94.78	95.82	127.54	122.11	135.43	124.72	127.45
4 HGA_3 HIGH	91.13	90.04	91.24	100.94	93.34	136.07	136.68	142.59	131.84	136.80
4 HGA_3 MEDIUM	71.28	80.99	68.23	73.12	73.41	124.99	138.96	122.85	128.46	128.82
4 HGA_3 LOW	72.80	90.81	79.28	65.78	77.17	146.13	136.70	143.36	129.08	138.82
15 HGA_3 HIGH	107.90	110.72	109.49	94.89	105.75	157.87	157.09	143.51	137.28	148.94
15 HGA_3 MEDIUM	76.81	100.57	97.55	102.83	94.44	130.77	171.35	145.23	154.86	150.55
15 HGA_3 LOW	93.99	95.26	101.92	86.02	94.30	147.46	149.20	146.89	138.29	145.46
30 HGA_3 HIGH	123.39	105.62	122.70	119.12	117.71	149.82	134.54	160.07	158.74	150.79
30 HGA_3 MEDIUM	90.77	100.42	107.50	96.41	98.78	167.33	158.53	158.35	146.66	157.72
30 HGA_3 LOW	108.21	85.82	95.47	62.65	88.04	165.29	129.17	152.55	106.14	138.29
200 HGA_3 HIGH	131.34	116.14	126.89	125.39	124.94	173.05	151.45	153.19	152.69	157.60
200 HGA_3 MEDIUM	91.16	96.16	109.15	107.44	100.98	148.11	159.55	158.13	167.35	158.29
200 HGA_3 LOW	104.68	70.17	84.34	95.00	88.55	155.34	122.28	135.68	149.27	140.64

Rep 1

	SEEDLING DRY MASS (mg)				No. of seedlings for dry mass				Dry mass per seedling (mg)				
	1	2	3	4	1	2	3	4	1	2	3	4	Average
CONTROL HIGH	438.40	501.50	438.00	449.50	24	25	25	25	18.27	20.06	17.52	17.98	18.46
CONTROL MEDIUM	469.20	449.00	406.10	440.70	23	24	22	23	20.40	18.71	18.46	19.16	19.18
CONTROL LOW	941.00	890.10	900.10	869.80	25	24	24	24	37.64	37.09	37.50	36.24	37.12
GA_3 HIGH	511.20	433.87	593.20	417.60	25	24	25	25	20.45	18.08	23.73	16.70	19.74
GA_3 MEDIUM	397.60	399.80	455.80	470.20	23	23	22	23	17.29	17.38	20.72	20.44	18.96
GA_3 LOW	884.00	855.10	813.00	825.10	24	24	22	23	36.83	35.63	36.95	35.87	36.32
4 HGA_3 HIGH	621.70	574.00	591.60	551.30	24	25	25	24	25.90	22.96	23.66	22.97	23.87
4 HGA_3 MEDIUM	427.00	497.20	453.90	486.20	25	25	23	24	17.08	19.89	19.73	20.26	19.24
4 HGA_3 LOW	927.40	984.60	993.50	824.50	24	24	24	22	38.64	41.03	41.40	37.48	39.63
15 HGA_3 HIGH	399.50	408.80	568.30	510.40	25	25	25	24	15.98	16.35	22.73	21.27	19.08
15 HGA_3 MEDIUM	423.60	439.10	482.50	439.10	25	25	25	24	16.94	17.56	19.30	18.30	18.03
15 HGA_3 LOW	873.80	970.10	909.80	870.20	24	23	25	25	36.41	42.18	36.39	34.81	37.45
30 HGA_3 HIGH	540.90	522.90	555.00	579.50	25	24	25	25	21.64	21.79	22.20	23.18	22.20
30 HGA_3 MEDIUM	459.50	491.50	529.30	428.20	21	23	25	25	21.88	21.37	21.17	17.13	20.39
30 HGA_3 LOW	938.10	800.20	899.30	950.90	25	24	25	25	37.52	33.34	35.97	38.04	36.22
200 HGA_3 HIGH	526.30	548.10	547.30	578.20	25	24	24	23	21.05	22.84	22.80	25.14	22.96
200 HGA_3 MEDIUM	385.40	439.80	471.10	516.50	22	22	24	22	17.52	19.99	19.63	23.48	20.15
200 HGA_3 LOW	987.50	924.40	951.40	880.00	24	24	24	24	41.15	38.52	39.64	36.67	38.99

Rep 1

	NORMALS					ABNORMALS					DEAD SEEDS				
	1	2	3	4	Average	1	2	3	4	Average	1	2	3	4	Average
CONTROL HIGH	24	24	25	24	97	0	1	0	1	2	1	0	0	0	1
CONTROL MEDIUM	21	23	18	19	81	2	1	4	4	11	2	1	3	2	8
CONTROL LOW	22	19	22	22	85	3	5	2	2	12	0	1	1	1	3
GA_3 HIGH	25	24	23	21	93	0	0	2	4	6	0	1	0	0	1
GA_3 MEDIUM	22	22	22	22	88	1	1	0	1	3	2	2	3	2	9
GA_3 LOW	20	19	19	22	80	4	5	3	1	13	1	1	3	2	7
4 HGA_3 HIGH	24	22	24	22	92	0	3	1	2	6	1	0	0	1	2
4 HGA_3 MEDIUM	23	25	20	24	92	2	0	3	0	5	0	0	2	1	3
4 HGA_3 LOW	24	22	23	15	84	0	2	1	7	10	1	1	1	3	6
15 HGA_3 HIGH	24	24	20	23	91	1	1	5	1	8	0	0	0	1	1
15 HGA_3 MEDIUM	25	23	25	22	95	0	2	0	2	4	0	0	0	1	1
15 HGA_3 LOW	22	22	23	21	88	2	1	2	4	9	1	2	0	0	3
30 HGA_3 HIGH	25	23	22	20	90	0	1	3	5	9	0	1	0	0	1
30 HGA_3 MEDIUM	20	23	23	22	88	1	0	2	3	6	4	2	0	0	6
30 HGA_3 LOW	23	23	24	25	95	2	1	1	0	4	0	1	0	0	1
200 HGA_3 HIGH	24	23	21	19	87	1	1	3	4	9	0	1	1	2	4
200 HGA_3 MEDIUM	21	21	22	19	83	1	1	2	3	7	3	3	1	3	10
200 HGA_3 LOW	21	22	24	22	89	3	2	0	2	7	1	1	1	1	4