A COMPARISON OF THE RELATIVE EFFECTIVENESS OF HIGH AND ULTRA HIGH DILUTIONS OF ABSCISIC ACID PREPARED BY SERIAL DILUTION AND SUCCUSSION AS OPPOSED TO DILUTIONS PREPARED BY SERIAL DILUTION ALONE, ON THE SYNTHESIS OF α-AMYLASE IN BARLEY ENDOSPERM HALF-SEEDS.

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I, Rouen Bruni, do hereby declare that this dissertation represents my own work both in concept and execution.

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Dedicated to my wife, Vanessa Bruni, and our daughter Mireia; and with thanks to Jesus Christ

"Unless an ear of wheat falls to the ground and dies, it remains only a single seed. But if it dies, it produces many seeds." John 12:24

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ABSTRACT

The aim of this study was to investigate the effects of high and ultra-high dilutions (ranging from 10^{-8} to 10^{-400}) of Abscisic acid (ABA) on the synthesis of α-amylase in barley seed endosperm halves (*Hordeum vulgare* Stirling, ex Caledon, Western Cape, South Africa, 1999 harvest), in order to determine whether these dilutions are able to produce biological effects, as homoeopathic theory would maintain they are (Gaier,1991:445-447).

A further aim of this study was to evaluate the role of succussion in the preparation of homoeopathic medicines. Central to the preparation of homoeopathic medicines is the principle of potentisation, which is a method of dilution that is unique to homoeopathy. It involves serial dilution with intervening mechanical agitation, called succussion, between each dilution level (Kayne, 1997:49). At each progressive stage of dilution the concentration of the solute diminishes, often beyond the point at which Avogadro's dilution limit of 6.022 X 10^{23} mol^{-1} is exceeded, so that theoretically no solute molecules remain in the solution (Gaier, 1991:47-48). Homoeopathic theory maintains that remedies thus prepared do not lose their therapeutic power in the process of dilution, but that due to the intervening succussion, their efficacy is in fact enhanced. Hence succussion is considered to be the process that sets homoeopathic dilutions apart from simple dilutions, Kayne (1997:49) states that, "This agitation is vitally important to the therapeutic efficacy of the remedy; dilution alone is not sufficient to produce the phenomenon".

In order to evaluate the role of succussion this study utilised five centesimal serial dilutions; the 4^{th}, 9^{th}, 15^{th}, 30^{th} and 200^{th}, which represent deconcentrations ranging from 10^{-8} to 10^{-400}, which dilution levels also span Avogadro's dilution limit. One series was prepared by diluting the ABA with intervening succussion and the other series was prepared by diluting the ABA without intervening succussion. The effects of these two methods on the production of α-amylase were then compared.

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An additional aspect of homoeopathic theory and practice that this study investigated was the principle of isopathy, which maintains that homoeopathically prepared dilutions of substances that directly cause a specific effect will counteract that effect (Gaier 1991:290). The isopathic principle was tested by using homoeopathically prepared high and ultra–high dilutions of ABA, a known inhibitor of α-amylase production and thus of germination, and noting whether they stimulated α-amylase production, as isopathic theory would predict.

Barley seeds which have been cut transversely in half, with the embryo-half discarded do not produce α-amylase, because of the absence of GA, which is usually provided by the embryo. However, if GA is added to the endosperm half-seeds, they do produce α-amylase in a dose dependent manner (Nissen, 1988). It has, therefore, been accepted that the presence of α-amylase is a specific and sensitive indicator of the presence of Gibberellic acid (Chrispeels and Varner, 1967, Cairns and de Villiers, 1986, Nissen, 1988, Steele, 1999) and of the initiation of germination.

The plant growth regulator substance abscisic acid (ABA) functions to inhibit seed germination in many species (Bewley and Black, 1994:106-108), it does this by blocking the increase in transcription of α-amylase genes, which is normally induced by the plant growth regulator Gibberellic acid (GA) (Roberts and Hooley, 1998:158). α-Amylase is an enzyme responsible for the digestion of starch reserves in the seed, which provides the energy needed for germination to proceed. Seeds in which none of the germination processes are taking place are said to be "dormant" or "quiescent". Dormant seeds are alive, they respire and in some cases grow very slowly, but they are in a state of rest with a low moisture content and virtually no metabolic activity (Bewley and Black, 1994:2). Of the many factors that mediate the processes of germination and dormancy, growth regulator events play the principal regulating role (Jann and Amen, 1977:15).

Two series of experiments were conducted, using two methods of preparation. The first

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method consisted of serial dilutions, with intervening succussion, of a range of potency levels, i.e. 4cH (10^{-8}), 9cH (10^{-18}), 15cH (10^{-30}), 30cH (10^{-60}) and 200cH (10^{-400}). The second method consisted of serial dilutions of a range of potency levels without intervening succussion, i.e. 4cD (10^{-8}), 9cD (10^{-18}), 15cD (10^{-30}), 30cD (10^{-60}) and 200cD (10^{-400}). Each series consisted of five replications and each replication consisted of 5 groups of 20 half-seeds treated with the different dilutions of ABA, and a sixth, control, group of 20 half-seeds treated with the extraction buffer only. Thus each series consisted of 30 groups comprising 600 half-seeds, resulting in 60 groups comprising a total of 1200 half-seeds.

An α-amylase activity standard curve was determined, based on pure α-amylase (1800 enzyme units/mg).

Each group of 20 half-seeds was weighed, placed in a 9cm petri dish lined with two No. 1 Whatman filter papers, and then moistened with 5ml of the various treatment solutions and incubated for 48 hours in a dark incubation cabinet set at a constant temperature of 15°C. After incubation the seeds were placed in a freezer at -5°C for 2 hours to halt hydrolysis of starch. When thawed each group of 20 seeds were macerated and then incubated with 10ml of extraction buffer in a shaker bath set at 30°C for 60 minutes, and then the extract was filtered.

The enzyme assay was prepared by incubating the filtrate with a dye-labelled substrate (PHADEBAS Amylase Test tablets) in a shaker-bath set at 50°C for 10 minutes, followed by filtration. The absorbance of the filtrate at 620nm was measured spectrophotometrically as a measure of the enzyme activity of each sample.

The data obtained was analysed by means of Univariate Analysis of Variance (Genstat for Windows). The results showed that unsuccussed ultra high dilutions of ABA are biologically active. Furthermore they showed a significant difference between the effects of succussed

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and unsuccussed dilutions of ABA. The unsuccussed dilutions produced less α-amylase than the control dilutions, which indicates that the inhibitory effects of ABA were still present in these dilutions. The succussed dilutions produced amounts of α-amylase that were not significantly different from those produced by the control. Statistically significant differences were noted between treatment groups and control groups, although not between the group of succussed dilutions when compared with the control. There were significant differences between the two methodologies employed, but not between the different dilution levels tested. All the results were calculated at the $\alpha = 5$ % level of significance.

The conclusion was drawn that ultra high dilutions can have biological effects and that succussion does alter the action of homoeopathically prepared medicines. The accepted chemical proposition that ultra high dilutions cannot have biological effects because, according to Avogadro's hypothesis, there are none of the original molecules present, was also challenged by the results of this study.

The experiments further served to demonstrate the suitability of the barley endosperm half-seed-α amylase system as an experimental model for investigating ultra high dilutions, as asserted by Steele (1999) earlier.

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TABLE OF ABBREVIATIONS

ABA – Abscisic acid

GA - Gibberllic acid

CH – Dilutions produced with succussion

CD – Dilutions produced without succussion

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THE DEFINITION OF TERMS

Germination

Seed germination is defined as the process that begins with the uptake of water by the seed (imbibition) and ends as the embryonic axis, usually the radicle, starts to elongate. (Bewley and Black 1994:1)

Dormancy

Seeds in which none of the germination processes are taking place are said to be "quiescent" or "dormant". These seeds are in a state of rest with a low moisture content (usually about 5 - 15%) and with virtually no metabolic activity (Bewley and Black, 1994:2).

Plant growth regulator

These are organic compounds produced in one part of a plant and transported to another part, where they elicit a response.

Law of similars

A fundamental principle of homoeopathy that maintains that a substance which is capable of provoking symptoms in a healthy organism acts as a curative agent in a diseased organism in which the same symptoms are manifested (van Wijk and Wiegant, 1994:12).

Ultra high dilution

Standardised aqueous or aqueous-alcoholic solutions where a substance has been diluted

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through a special dilution process in such a way that the concentration ratio of solute to solvent becomes of the order of Avagadro's number ($6.022 \times 10^{23} mol^{-1}$) or below (Endler and Schulte 1994:1).

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 opposite hand (Kayne 1997: 49).

Isopathy

The use of the same and not merely the similar as medicines for treating disease (Gaier 1991:290).

Avogadro's number

A formulation demonstrated by Amedeo Avogadro (1776-1856) that in one mole of any substance there are 6.022×10^{23} molecules. The implication of this is that there is a limit to the number of serial dilutions that can be made without losing the original substance altogether. Theoretically, solutions diluted beyond Avogadro's number have no molecules of the original solute remaining in them. Homoeopathically this point is reached when potencies pass the 12CH (10^{-24}) level. (Kayne, 1997:27)

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Our dependence on agriculture is an issue most farmers would have us consider more seriously, and agriculture itself is dependent on the viability and germinability of the seed it uses.

Seeds in which none of the germination processes are taking place are said to be "dormant" or "quiescent". Dormant seeds are alive, they respire and in some cases grow very slowly, but they are in a state of rest with a low moisture content and virtually no metabolic activity (Bewley and Black, 1994:2). Many factors mediate the processes of germination and dormancy, these can be classified as either internal or external controls. External controls relate to the seeds environment and internal controls include anatomical, genetic metabolic and hormonal events, of which hormonal events play the principal regulating role (Jann and Amen, 1977:15).

This study investigated the effects of one particular plant growth regulator on seed germination. The growth regulator abscisic acid (ABA) functions to inhibit seed germination in many species (Bewley and Black, 1994:106-108), it does this by blocking the increase in transcription of α-amylase genes, which is normally induced by the plant growth regulator Gibberellic acid (GA) (Roberts and Hooley, 1988:158). α-Amylase is an enzyme responsible for the hydrolysis of starch reserves in the seed, which provides the energy needed for germination to proceed.

The aim of this study was to investigate the effects of high and ultra-high dilutions (ranging from 10^{-8} to 10^{-400}) of ABA on the synthesis of α-amylase in the aleurone layer of barley

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endosperm half-seeds, in order to determine whether these dilutions are able to produce biological effects, as homoeopathic theory would maintain they are. Barley seeds which have been cut transversely in half, with the embryo-half discarded do not produce α-amylase, because of the absence of GA, which is usually provided by the embryo. However, if GA is added to the endosperm half-seeds, they do produce α-amylase in a dose dependent manner (Nissen, 1988). It has, therefore, been accepted that the presence of α-amylase is a specific and sensitive indicator of the presence of Gibberellic acid (Chrispeels and Varner, 1967, Cairns and de Villiers, 1986, Nissen, 1988, Steele, 1999) and of the initiation of germination.

A further aim of this study was to evaluate the role of succussion in the preparation of homoeopathic medicines. In order to do this the study utilised five centesimal serial dilutions; the 4^{th}, 9^{th}, 15^{th}, 30^{th} and 200^{th}, which represent deconcentrations ranging from 10^{-8} to 10^{-200}, which dilution levels also span Avogadro's dilution limit. One series was prepared by diluting the ABA with intervening succussion and the other series was prepared by diluting the ABA without intervening succussion. The effects of these two methods on the production of α-amylase were then compared.

An additional aspect of homoeopathic theory and practice that this study investigated was the principle of isopathy, which maintains that homoeopathically prepared dilutions of substances that directly cause a specific effect will counteract that effect (Gaier 1991:290). The isopathic principle was tested by using homoeopathically prepared high and ultra–high dilutions of ABA, a known inhibitor of α-amylase production and thus of germination, and noting whether they stimulated α-amylase production, as isopathic theory would predict.

This research was conducted on Barley seeds as numerous previous studies of the action of ABA have been conducted on barley as it is highly sensitive to ABA (Bewley and Black,1994: 296-304). In addition previous homoeopathic research has been conducted by Steele (1999) on the effect of Gibberellic acid, a germination-promoting hormone on the

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production of α-amylase in barley seeds. Commercially barley is also an important crop, with an annual global production exceeding 180 million tons, the majority of which is used for the production of malt in the manufacture of beer and spirits.

Central to the preparation of homoeopathic medicines is the principle of potentisation, which is a method of dilution that is unique to homoeopathy. It involves serial dilution with intervening mechanical agitation, called succussion, between each dilution level (Kayne, 1997:49). At each progressive stage of dilution the concentration of the solute diminishes, often beyond the point at which Avogadro's dilution limit of 6.022×10^{23} /mol is exceeded, so that theoretically no solute molecules remain in the solution (Gaier, 1991:47-48). Homoeopathic theory maintains that remedies thus prepared do not lose their therapeutic power in the process of dilution, but that due to the intervening succussion, their efficacy is in fact enhanced. Hence succussion is considered to be the process that sets homoeopathic dilutions apart from simple dilutions, Kayne (1997:49) states that, "This agitation is vitally important to the therapeutic efficacy of the remedy; dilution alone is not sufficient to produce the phenomenon".

The proposition that homoeopathic medicines are actually rendered more vigorous in their action the more they are diluted has been the cause of much contention and not little derision from scientists. However, modern homoeopathic researchers maintain that due to the action of the intervening succussion on the dilutions, these undergo subtle physical changes, and that homoeopathic medicines consequently act by means of biophysical rather than biochemical interactions (Resch and Gutmann, 1991:191-213 and Towsey and Hasan, 1995). There are numerous theories that propose possible mechanisms for the biophysical action of homoeopathic medicines. However, all these theories are hampered by the same difficulty i.e. the lack of experimental models and equipment sensitive enough to detect and measure the subtle changes in structure and effect that succussion is proposed to produce in homoeopathic potencies.

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Homoeopathic agricultural research is a relatively recent phenomenon and several studies are particularly relevant to this research. Betti *et al* (1994:195-201) conducted a randomised laboratory trial where the effects of various homoeopathic dilutions of *Arsenicum album* (Arsenic trioxide) on germination were tested. Three treatment groups showed a significance level of less than 1% and another was below 5%, and the results showed that differences between the treatment groups could not be explained as a mere effect of intrinsic seed variability. Hopkins (1998) investigated the biological effects of homoeopathic treatments of Sulphur, Nitric acid and Camphor on lettuce seed germination. He found statistically significant differences between the treatments and recommended that germinability studies using plant hormones prepared homoeopathically be conducted.

Steele (1999) conducted a study of the effects of homoeopathic dilutions of Gibberellic acid on the synthesis of α-amylase in barley endosperm half-seeds. He also compared remedies prepared by serial dilution with intervening succussion as opposed to remedies prepared without succussion. He found that homoeopathic dilutions of GA were capable of causing statistically significant α-amylase synthesis, but he found no difference between the effects of dilutions prepared with succussion or without succussion. He concluded that succussion is not a significant factor in the efficacy of homoeopathic medicines. He recommended that other plant hormones be used in similar experimental models and that more studies be conducted to compare succussed and unsuccussed medicines.

Christie (1995) undertook a study with the aim of investigating the relevance of succussion in the preparation of homoeopathic medicines. She prepared the homoeopathic medicines *Pulsatilla, Kalium carbonicum* (Potassium carbonate), *Natrum muriaticum* (Sodium chloride) and *Psorinum*, comparing the effects of no succussions with various numbers of succussions administered either by hand or by machine. The medicines were then added to *Saccharomyces cerevisiae* cultures and the growth of the yeast was measured. She found that succussion played no role and hence that the relevance of succussion in the preparation of homoeopathic medicines was questionable. She recommends further *in vitro* testing of

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homoeopathic medicines to assess the relevance of succussion.

Within the field of homoeopathic research, this study extends those mentioned above. In particular it builds on recommendations by Steele (1999) that future homoeopathic research use unsuccussed medicines as a further control. Steele's suggestion that other plant hormones be investigated for their effects on germination was also satisfied in that the effects of homoeopathic dilutions of Abscisic acid on germination were studied. The unique contribution of the present study was to investigate the isopathic principle, in that a plant hormone responsible for the suppression of germination was studied in order to detect any germination promoting properties when this same hormone was prepared in ultra-high homoeopathic dilutions.

1.2 Aim of the study

The aim of this study was to compare the relative effectiveness of high and ultra high dilutions of abscisic acid (ABA) prepared by serial dilution and succussion, as opposed to high and ultra high dilutions of ABA prepared by serial dilution alone, on the synthesis of α-amylase enzyme in de-embryonated barley (*Hordeum vulgare*) endosperm half-seeds, in terms of enzyme units produced per gram fresh weight of endosperm halves.

1.3 Statement of the objectives

1.3.1 The first objective

The first objective was to determine the effect of a range of succussed serial dilutions of ABA on the production of α-amylase enzyme in barley endosperm half-seeds.

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1.3.2 The second objective

The second objective was to determine the effect of a range of unsuccussed serial dilutions of ABA on the production of α-amylase enzyme in barley endosperm half-seeds.

1.3.3 The third objective

The third objective was to compare the effects of the two methods of preparation of ABA on the production of α-amylase in barley endosperm half-seeds.

1.4 Statement of the hypotheses

All hypotheses are stated in the null form.

1.4.1 The first hypothesis

It is hypothesised that succussed serial dilutions of Abscisic acid will have no statistically significant effect on the synthesis of α-amylase in barley endosperm half-seeds.

1.4.2 The second hypothesis

It is hypothesised that unsuccussed serial dilutions of Abscisic acid will have no statistically significant effect on the synthesis of α-amylase in barley endosperm half-seeds.

1.4.3 The third hypothesis

It is hypothesised that there will be no significant difference between the two methods of dilution i.e. with or without succussion, on the synthesis of α-amylase in barley endosperm

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half-seeds.

1.4.4 The fourth hypothesis

It is hypothesised that there will be no difference between the levels of dilution on the production of α-amylase in barley endosperm half-seeds.

1.5 The significance of the study

The results of this study have shown that ultra high dilutions can have significant biological effects, this serves to add weight to the contention of many homoeopathic researchers and practitioners that ultra high dilutions are able to elicit biological effects, and that the action of homoeopathic medicines needs to be understood in terms other than mere placebo or suggestion. In addition it has demonstrated a significant difference between remedies that are prepared by dilution with succussion and those prepared without succussion. Although it can not be claimed that the succussed dilutions were more effective than the unsuccussed, there was, however, a significant difference between the effects of the succussed and unsuccussed dilutions. Further study may show that this is in support of homoeopathic theory that maintains that succussion is an essential element in the preparation of homoeopathic medicines, and that remedies prepared with succussion are distinctly different from those prepared without it. However, one aspect of homoeopathic theory that is challenged by this study is the principle of isopathy, as homoeopathic dilutions of a germination inhibiting substance were not found to stimulate germination, but rather continued to suppress germination, even at dilutions of 10^{-400}. Furthermore, as the seed based study eliminates the potential for placebo or suggestion effects, this study demonstrates that homoeopathic medicines can have intrinsic effects, which cannot be ascribed to placebo effects or suggestion.

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1.6 The implications of the study

The most important implications of this study relate to the role of succussion in the preparation of homoeopathic remedies as well as the relevance of chemical theories regarding the effectiveness of ultra high dilutions. The results of this study demonstrate a difference between remedies prepared by serial dilution and succussion and those prepared by serial dilution without intervening succussion. It could be hypothesised that the inhibitory action of ABA was inhibited by the process of succussion, however, the conclusion was drawn that ultra high dilutions can have biological effects and that succussion does alter the action of homoeopathically prepared medicines. This may be interpreted to support homoeopathic theory that maintains that a substance, which, in material doses has a specific effect, will in homoeopathic dilution, i.e. serial dilution with intervening succussion, counteract that effect. Homoeopathic theory maintains that succussion is an essential element in the preparation of homoeopathic medicines, as it results in the production of a medicine that has biological effects, which differ significantly from those of unsuccussed dilutions of the same substance.

This study also challenges the chemical concept of Avogadro's constant which maintains that dilutions in excess of 6.022×10^{-23} cannot have biological activity, as the study demonstrated biological activity in dilutions up to 10^{-400}. Therefore, it challenges chemical theorists to reconsider this theory and to propose hypotheses that account for the biological activity observed in this study.

1.7 The benefits of the study

Besides demonstrating the effectiveness of ultra high dilutions, this study confirmed the work done by Steele (1999), which demonstrated that the barley endosperm, α-amylase experimental model is a suitable one for studying the effects of ultra high dilutions. The fact that the preparation procedures are standardised and that the objective outcome

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measurements ensure reliability, as well as the direct relationship between treatment and effect, ensuring validity, demonstrate its effectiveness as an experimental model. Furthermore, this experimental model avoids the ethical issues involved in animal and human studies and can be completed in two to three weeks in a standard laboratory.

In addition, activity of ABA at ultra high dilutions has potential economic benefits if these dilutions are able to be employed effectively in the prevention of pre-harvest sprouting of wheat, which results in substantial losses to the agricultural industry annually, as the utilisation of higher concentrations of ABA is not economically viable. In addition, as studies have suggested possible benefits from being able to manipulate stomatal opening in plants, including; decreased water consumption, protection from chilling injury and reduced pollutant uptake (Roberts and Hooley, 1988:169); and as ABA has been implicated in the regulation of stomatal closure, economical benefits may result if ultra high dilutions of ABA are able to demonstrate stomatal regulating effects.

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CHAPTER TWO

REVIEW OF THE RELATED LITERATURE

2.1 Introduction

The focus areas of this research are seed germination, seed dormancy, barley seeds, plant hormones, Abscisic acid, succussion and homoeopathic agricultural research. This review will attempt to cover each of these aspects and their relevance to the study undertaken.

2.2 Seed Germination

Mankind, indeed all animal forms of life, depend on the energy conversion undertaken by plants to a degree that we seldom contemplate. The ability of plants to survive and perpetuate themselves is dependent on the success with which they are able to reproduce and a vital part of this process is the germination of their seeds.

Seed germination is the process whereby the embryo resumes the growth activities that were suspended during quiescence or dormancy, and during which new genetic programs are initiated. Of the many factors involved in germination, including anatomical, genetic and metabolic events; hormonal events are the primary agents (Jann and Amen 1977:8-15).

Because of the commercial implications of our dependence on agriculture, and the vital role seed germination plays in agriculture as well as in industrial processes, e.g. malting, much research has been undertaken into seed germination and the factors that control it.

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2.3 Seed Dormancy

Seed germination begins with the uptake of water by the seed and ends as the embryonic axis, usually the radicle, starts to elongate (Bewley and Black, 1994:1). Seeds in which none of the germination processes are taking place are said to be "quiescent" or "dormant". There is a distinction made between dormancy which is imposed by unfavourable environmental conditions, such as low temperature or insufficient moisture, called "quiescence", "imposed dormancy" or "temporary dormancy"; and "true dormancy", which refers to a state of temporarily suspended growth which is due to inhibitory internal conditions, also called "innate dormancy" or "rest" (Moore, 1979:181-182).

The origin of dormancy is to be found in the phase of the seed's development. As the seed develops a point is reached at which its development into a seedling is arrested because of dehydration, this is called "developmental arrest". At this stage seeds may be either dormant (true dormancy) or non-dormant. The dormant seeds will not germinate, not even in conditions that are perfectly adequate for germination. The non-dormant seeds will only germinate if factors required for germination are present. These are factors such as adequate water, light, soil components or diurnal temperature fluctuations. If these factors are not present germination is passively inhibited and the seeds are said to be in a state of "temporary dormancy". If this inhibition of germination is prolonged the seeds may gradually enter a state of secondary dormancy that resembles primary dormancy (see figure 2.1 - overleaf) (Karssen 1995:336).

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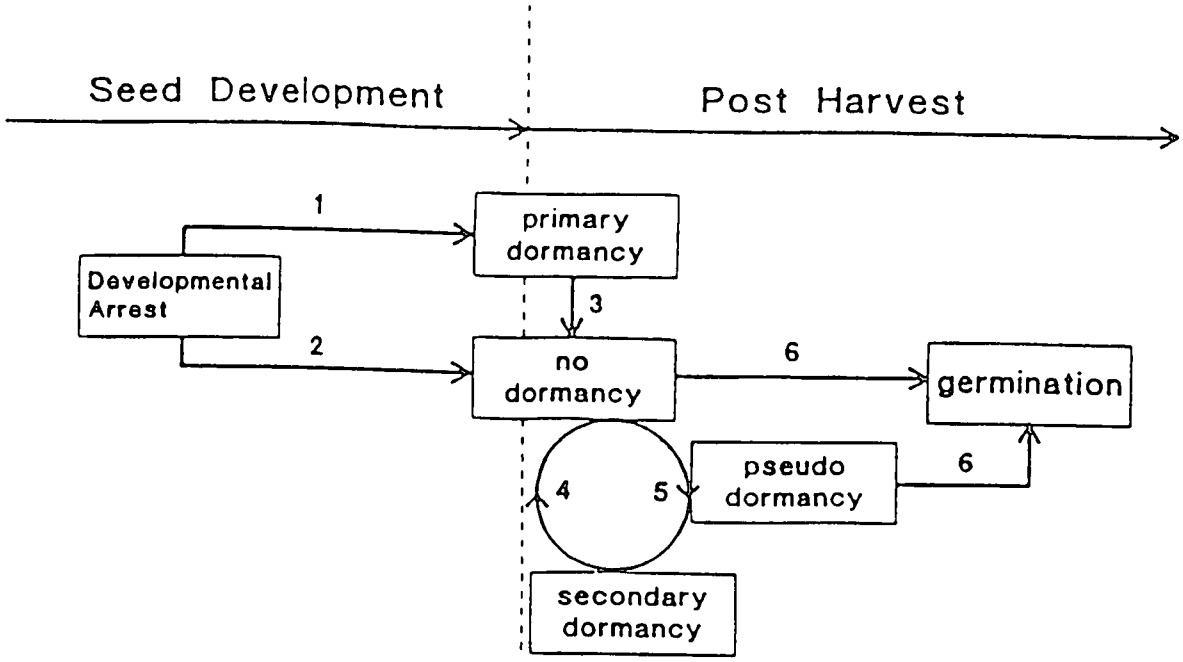

Figure 2.1 Schematic presentation of transitions between different states of dormancy and germination. The dotted line represents the phase during development in which seeds are dehydrated.

These dormant seeds are alive, they respire and in some cases grow very slowly, but they are in a state of rest, with a low moisture content (usually about 5 - 15%) and with virtually no metabolic activity (Bewley and Black, 1994:2). It has been found that in the dormant seed the genetic material (the DNA) is completely or nearly completely repressed. So the process of DNA transcription, the production of messenger RNA, and the production of enzymes and structural proteins essential for growth and metabolism, are all repressed (Moore, 1979:202). What is remarkable is that often after many years such dormant seeds are able, given suitable conditions, to resume a high level of metabolism and to germinate.

It may seem questionable, since the function of a seed is to germinate and produce a new

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plant that dormancy, which essentially is a barrier to germination, exists at all. But there are situations in which it would not be beneficial for a seed to germinate, e.g. conditions of drought or temperature extremes - environmental conditions that would be adverse or lethal to plants in an active state of growth; and so dormancy is of important biological significance (Moore, 1979:181). Dormancy also plays a role in ensuring that seed germination is distributed in time as well as in space, thus allowing for seeds to germinate in environments of reduced competition (Bewley and Black, 1994:199-201).

2.4 Plant Growth Regulator Substances

There are many factors involved in the processes of germination and dormancy, classified as either internal or external controls. The external controls are factors relating to the seed's environment, e.g. temperature, water and oxygen availability, light quality and quantity as well as the absence of noxious chemicals. Yet, in many cases all of these conditions may be satisfied but the seed fails to germinate. This is because there exists within the seed itself some block that must be overcome before germination can proceed, these are the internal controls, which include anatomical, genetic, metabolic and hormonal events. Of these, hormonal events have been found to play the principal regulating role with respect to germination (Jann and Amen, 1977:15, Bewley and Black, 1994:199).

Plant growth regulator substances are organic compounds that are produced in one part of the plant and then transported to another part where they then elicit specific biochemical, physiological and/or morphological responses (Moore *et al.* 1995:411). There are five major classes of growth regulators in plants, also called plant hormones; the gibberellins, auxins, the cytokinins, ethylene and abscisic acid. These all have a wide range of effects and are effective in minute concentrations, as low as $<1\mu M$, (Moore 1979:28).

The most extensively investigated plant growth regulating substances are the gibberellins, of which Gibberellic acid is the most important. It has been found to be essential for

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germination to progress (Karssen, 1995:346). The gibberellins stimulate germination by stimulating the production of α-amylase in the aleurone layer which leads to the hydrolysis of starch in the endosperm, producing sugar which is available as an energy source for the germinating seedling (Moore, 1995:423). Ethylene plays a role in the regulation of gene expression involved in fruit ripening (Roberts and Hooley, 1988: 158-159) and the cytokinins are involved in dormancy breaking (Roberts and Hooley, 1988:168-169).

2.5 Abscisic Acid

The plant growth regulator abscisic acid (ABA) was the focus of this research. ABA functions to inhibit germination in many seed species (Black, 1991:115, Bewley and Black, 1994:106-108, Jann and Amen, 1977:21). In the seeds of cereals ABA inhibits germination by inhibiting the Giberrellic acid (GA) induced synthesis of α-amylase in the aleurone layer, it does this by blocking the increase in the transcription of α-amylase genes that is normally induced by GA (Roberts and Hooley, 1988:158). Studies confirming the pivotal role that ABA plays in dormancy induction have involved comparing dormancy induction in seeds containing ABA with dormancy induction in ABA-deficient mutants of the same species. Particularly well documented are studies on dormancy induction in the weed *Arabidopsis thaliana* which has mutants that are ABA-deficient (Karssen, 1995:338). These studies have shown that the onset of dormancy requires the presence of ABA, as dormancy was absent in the ABA-deficient mutant of this weed. Furthermore, it has been found that dormancy induction not only depends on the presence of ABA, but is also dependent on the sensitivity of the seed to ABA (Bewley and Black, 1985:118-119,Karssen, 1995:338). In a study on yellow cedar seeds, in which they underwent dormancy breaking treatments, Schmitz *et al.* (2000: 1159-1162) found that seeds which were treated with the dormancy breaking treatments experienced an approximately 2-fold decrease in ABA content of the embryo, but that the embryo simultaneously experienced a 10-fold lowered sensitivity to ABA. This clearly demonstrates that a decrease in the ABA content within the seed is not sufficient for dormancy breakage, but that the embryo also has to experience reduced sensitivity to the

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ABA which is present.

The free ABA content in seeds is high during development and is relatively low or even absent at maturity. Typically the ABA content is extremely low early in development but rises as development progresses, to reach a maximum at about one third to one half of the time from seed initiation to maturity, and then it falls to low levels (Karssen 1995:337). In many species, isolation of the embryo from the developing seed stops normal embryogenetic growth and allows precocious germination to occur. However, if ABA is added to the incubation medium, the isolated embryo is held in the embryogenetic mode (Karssen, 1995:338). This is further evidence of the importance of ABA in delaying germination, which is of economical importance especially in preventing the pre-harvest sprouting of wheat, which is very costly to the agricultural sector. In addition to its fundamental role in dormancy induction and maintenance, ABA also stimulates reserve protein deposition and plays a role in the acquisition of desiccation tolerance by seeds (Black, 1991:102-112).

2.6 Barley

Barley seeds were used in this study as many previous studies of the action of ABA have been conducted on these seeds, which are highly sensitive to the action of ABA (Bewley and Black, 1994:296-303). In addition previous research has been conducted in this department by Steele (1999), investigating the effect of homoeopathic dilutions of gibberellic acid (GA_3), a germination-promoting hormone, on barley seeds. This researcher considered it, therefore, to be of interest to evaluate the effects of homoeopathic dilutions of ABA, a germination-inhibiting hormone, on the germination process in barley seeds.

The variety of barley seed that was utilised in this study is *Hordeum vulgare* Stirling (from Caledon, Western Cape, 1999 harvest). Barley is a monocotyledon of the Graminales order, Gramineae family. It is estimated that the annual world production of barley exceeds 180 million metric tons. Of this a large proportion (+- 35-40%) is used in malting while much of

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the rest is used as feed for livestock (Bewley and Black, 1994:378). Malting barley is allowed to germinate under controlled conditions, producing a limited amount of seedling growth, it is then dried and lightly roasted. The function of malting is to achieve the production of enzymes within the barley seeds, which then hydrolyse the starch reserves of the endosperm to make sugars available for fermentation. The malt formed has many uses - malt extracts, syrups, breakfast foods, coffee substitutes - but most of it is used in the manufacture of beers and spirits such as whisky, gin and vodka (Bewley and Black, 1994: 378-381). It is clear that germination is an essential factor in the industrial process of malting and hence the viability and dormancy of barley seed is of critical importance to this huge industry.

2.7 α-Amylase production, barley seed germination and the role of Abscisic acid

The process of germination in barley seeds begins with the absorption of water by the seed, called "imbibition". In seeds that are not maintained in a state of dormancy by ABA, this imbibition leads to the release of gibberellins, germination-promoting hormones, by the embryo part of the seed. These gibberellins diffuse into a specialised layer of the endosperm, the aleurone layer, adjacent to the seed coat. In the aleurone layer these hormones stimulate transcription of genes of the hydrolytic enzymes α-amylase, β-amylase, the debranching enzymes and α-glucosidase. α-Amylase is the main enzyme produced. The initial production of α-amylase occurs in the region of the scutellum and in the few aleurone cells that penetrate the peripheral regions of the scutellum. The α-amylase is then released into the starchy endosperm where it catalyses the conversion of starch to sugar, to be used as an energy source for the rapid growth of the seedling (Bewley and Black, 1994:296-297)(See Figure 2.2).

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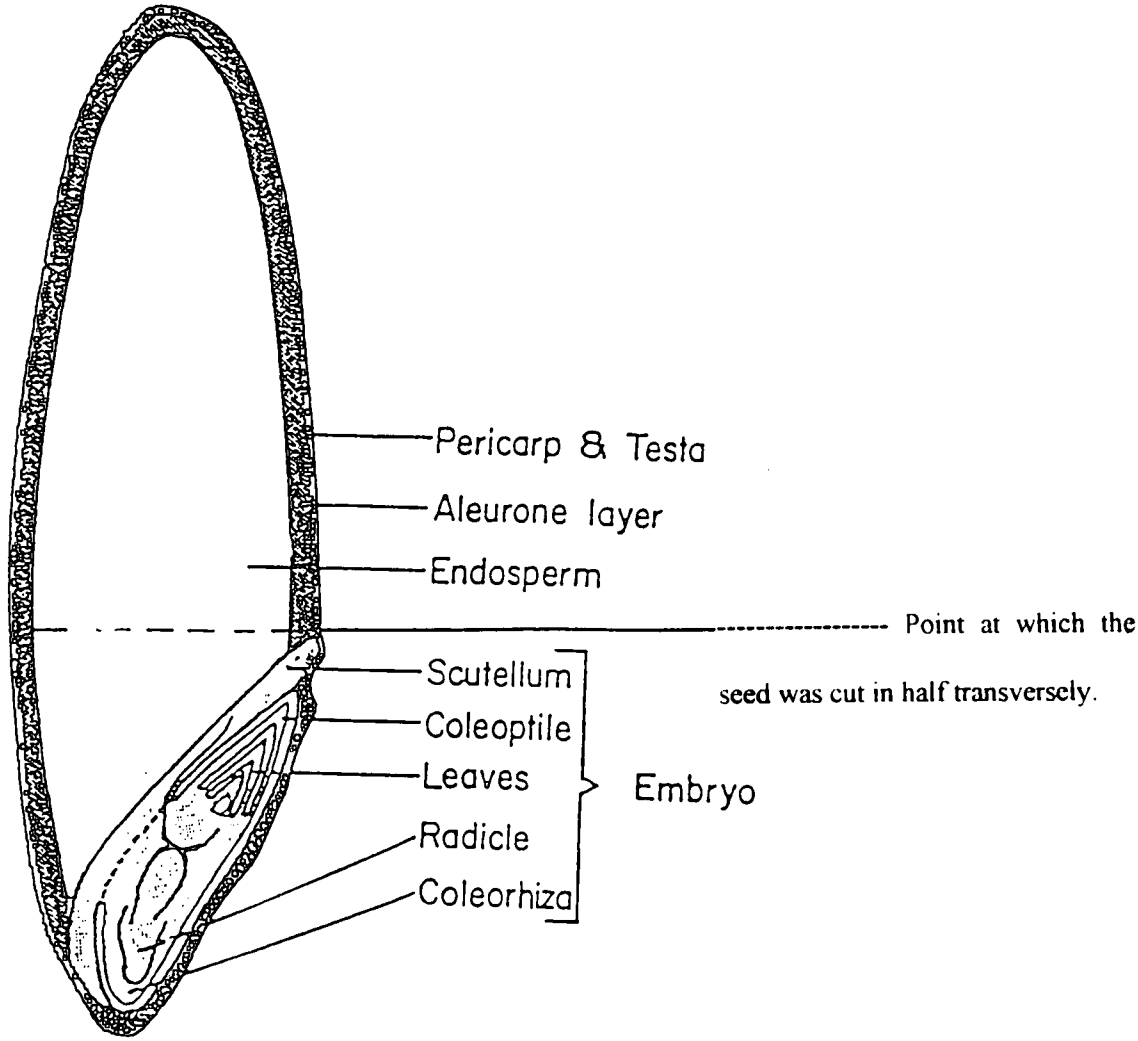

Figure 2.2 Longitudinal section of a barley seed (From Moore, 1981: 53)

α-Amylase is synthesised *de novo* in the endoplasmic reticulum (ER) of the cells of the aleurone layer. Two processes involved in this synthesis of α-amylase in the ER are stimulated by GA, i.e. the transcription of α-amylase mRNA and the transport of Ca^{2+} from the cytosol into the ER (Bush *et al.*1991). ABA obstructs the GA-induced production of α-amylase by blocking the transcription of α-amylase mRNA. The main function of α-amylase

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is to mobilise the stored carbohydrate reserves found in the endosperm so that the energy needed for germination will be made available. The form of carbohydrate reserve found in barley seeds is starch, which is laid down in discrete, predominantly spherical, subcellular bodies called starch grains. There are two main groups of starch grains – large and small grains. The small grains account for +- 90% of the total number of starch grains, but make up only 10% of the total starch by weight (Bewley and Black 1994:12-13, 296-7). There are several hydrolytic and phosphorolytic enzymes that are responsible for starch degradation and mobilization in barley, however, α-amylase is the only enzyme that is able to effectively degrade the native polymer structure, and it is, therefore, essential for the initial depolymerization of the starch granule. Once the polysaccharides have been released by the action of α-amylase they are able to be further degraded by a combination of α-amylase, β-amylase, starch phosphorylase and debranching enzymes (Ziegler, 1995:452). It is, therefore, apparent that without α-amylase production, digestion of stored starch, and hence germination, cannot proceed.

Studies have shown that barley seeds which have been cut in half with the embryo-half discarded do not produce α-amylase because of the absence of gibberellic acid, which is usually supplied by the embryo. If gibberellic acid is added to the endosperm half-seeds they do produce α-amylase in a dose dependent manner (Nissen, 1988). It has, therefore, been accepted that the presence of α-amylase is a specific and sensitive indicator of the presence of gibberellic acid (Chrispeels and Varner, 1967, Cairns and de Villiers, 1986, Nissen, 1988, Steele, 1999) and of the initiation of germination.

The presence of α-amylase is tested for by using Phadebas tablets, which, although originally developed to determine levels of human α-amylase, have been successfully used to determine α-amylase levels in cereals and cereal products (Barnes and Blakeney, 1974, Cairns and de Villiers, 1986). Each Phadebas tablet consists of a water-insoluble cross-linked starch polymer, derived from partially hydrolysed potato starch, carrying a blue dye. In the

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presence of α-amylase this is hydrolysed to form water-soluble blue fragments, which absorb light at 620nm (Anon 1994).

Experimental studies testing the response of biological systems to a particular treatment are generally very difficult to interpret because of the complexity of the system and the many factors, beyond the experimenters control, that come to bear on the system. However, the barley endosperm-α-amylase model reduces that complexity considerably as any synthesis of α-amylase can be related directly to the effect of the corresponding treatment. As this study utilised barley seeds of which the embryo-half had been discarded, and hence were unable to produce the GA essential for α-amylase production, the presence of any α-amylase detected by the Phadebas test would have to be explained as being a result of the administered treatment. This, therefore, renders this experimental model very suitable for investigating the action of high and ultra-high dilutions.

There are other advantages rendered by experiments involving seeds in homoeopathic and medical research; one does not have to deal with the complex ethical issues involved in experiments on human or animal subjects (Hopkins 1998), as well as the fact that the placebo effect is eliminated (Pelikan and Unger, 1971). Much of the criticism directed at homoeopathy is based on the assumption that most of the effects of homoeopathic medicine can be ascribed to the placebo effect (Reilly *et al.* 1986).

2.8 Hormones and Homoeopathy

To the knowledge of this researcher ABA has not been utilised in homoeopathic treatment, although other hormones are used to produce homoeopathic medicines known as "sarcodes". These are homoeopathic medicines made from healthy tissues or secretions of plants, animals or humans (Gaier, 1991:292). Currently used sarcodes produced from hormones include: Folliculinum (oestrogen), Parathyroidinum and Corticotrophin.

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Another development in homoeopathic practice is the use of isopathic medicines, which is a variation on the "Law of similars", a fundamental principle of homoeopathy that maintains that a substance, which is capable of provoking symptoms in a healthy organism, acts as a curative agent in a diseased organism in which the same symptoms are manifested (van Wijk and Wiegant, 1994:12). An example of isopathic treatment would be to administer homoeopathic dilutions of plasmodium parasites to a patient with malaria. Of interest to this researcher was to observe whether the isopathic principle could be demonstrated by administering ABA, an inhibitor of germination, in ultra high dilutions and noting whether it stimulated germination.

2.9 The preparation of homoeopathic medicines

2.9.1 Introduction

The preparation of homoeopathic medicines is undertaken in accordance with the foundational principle of homoeopathy, the so-called "Law of Similars", originally expressed as *Similia similibus curentur*, which translates as, "Let likes be cured by likes". This means that a substance which in high (material) doses is able to induce particular symptoms in a healthy person, will, if administered in minute doses, cure those symptoms when they present in an ill person (Gaier, 1991:264).

As well as the "Law of similars", there is another fundamental principle of homoeopathy that is central to the preparation of homoeopathic medicines, viz. the process of potentization. This is a method of dilution unique to homoeopathy, which is claimed to increase the strength of the medicine, hence the name "potentization" (Kayne, 1997:49). Potentization involves the process of serial dilution with intervening succussion, which is the vigorous agitation of the liquid dilution between each dilution level, by shaking where each stroke ends with a jolt, as by striking the vial against the heel of the opposite hand (Kayne, 1997:49).

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At each progressive stage of this concomitant serial dilution and succussion the concentration of the solute diminishes, in many cases up to and beyond the point at which Avogadro's dilution limit of 6.022 X 10^{23} /mol is exceeded, which occurs at the 12cH level. Therefore, theoretically, at dilutions higher than this it is expected that not one molecule of the original base substance remains (Gaier 1991: 47-48). The dilutions thus produced are frequently referred to in homoeopathic literature as "Ultra high dilutions", as opposed to the alternative term used in chemical and pharmacological research, "Ultra low concentrations/doses" (Doutremepuich, 1991). Although this difference may seem trivial, it is significant as it calls attention to the difference in the focus of the two areas of research. The term "Ultra high dilution" serves to emphasise the homoeopathic researchers' preoccupation with the role of the solvent, whereas "Ultra low concentration/dose" indicates an emphasis on the role of the solute. This use of terminology reflects the homoeopathic interest in biophysical effects above the biochemical effects that interest chemical and pharmacological researchers (Towsey and Hassan 1995).

2.9.2 The law of similars

Although the concept of similars as described above may seem a totally foreign one, it has begun to be investigated beyond the confines of homoeopathy and orthodox science has termed it "Hormesis" or the "Biphasic effect" (Cambar, 1994:5-18). Hormesis is defined as a stimulatory effect that occurs in biological systems after exposure to a low concentration of an otherwise toxic agent (Cambar, 1994:5). Another similar concept that has long been associated with homoeopathy is the *Arndt-Schulz law*, which states that, "Every stimulus on a living cell elicits an activity which is inversely proportional to the intensity of the stimulus" (Cambar, 1994:6). Examples of the application of the hormetic effect are to be seen in conventional medicine; a well known example is the use of Digitalis, which in large doses causes arrhythmic tachycardia, but in smaller doses is used to treat atrial fibrillation. Mercury salts also are used in small doses as diuretics, but in large doses they cause oliguria and

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anuria (Reynolds and Prasad 1982:541,581).

Another study demonstrating the hormetic effect and which is also of particular interest with regard to the employment of isopathic medicines is that of De Gerlache and Lans who investigated the effect of exposure to documented carcinogens in rats that had previously been exposed to homoeopathic potencies of the carcinogenic agents. Rats were fed 2-Acetylaminofluorene (2AAF) or Phenobarbital (PB) in the 9cH potency i.e. a molar concentration of +- 10^{-18} for 21 days and were then fed the same carcinogens in material doses for 12 months. Animals from the various groups were periodically sacrificed in order to be analysed for the development of liver cancer. The results showed that the 9cH solutions of both PB and 2AAF had no effect on the percentage of rats with macroscopic liver lesions, but that they significantly increased the percentage of animals surviving with such lesions. In both the PB and 2AAF groups, 100% of rats with lesions survived as compared to +- 60% in the control groups (Doutremepuich 1991:17-26).

However, although such reversed biological effects in low concentrations of agents that are toxic in high concentrations seem to correspond exactly to the *Similia* principle, it needs to be borne in mind that Hormesis is not homoeopathy, mainly because the classical hormetic systems act at concentrations that are much higher than those used in homoeopathy. At best it can be used as an explanation for the action of homoeopathic remedies at low potency, but it is very doubtful whether it is at all valid at the ultra low concentrations that are mainly used in homoeopathy (Popp, 1994:180). Homoeopaths, however, object that homoeopathic medicines are not merely dilutions, but also undergo the process of succussion at each stage of dilution, and that the hormetic effect is potentiated by the succussion such that it can act at far lower concentrations (Cambar, 1994:8).

2.9.3 The preparation of homoeopathic dilutions

Homoeopathic dilutions are prepared by stepwise serial dilutions in distilled water or alcohol.

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These dilutions are either in decimal (1:10), centesimal (1:100) or quinquagenimillesimal (1:50 000) scales, with succussion at every step (see figure 2.3).

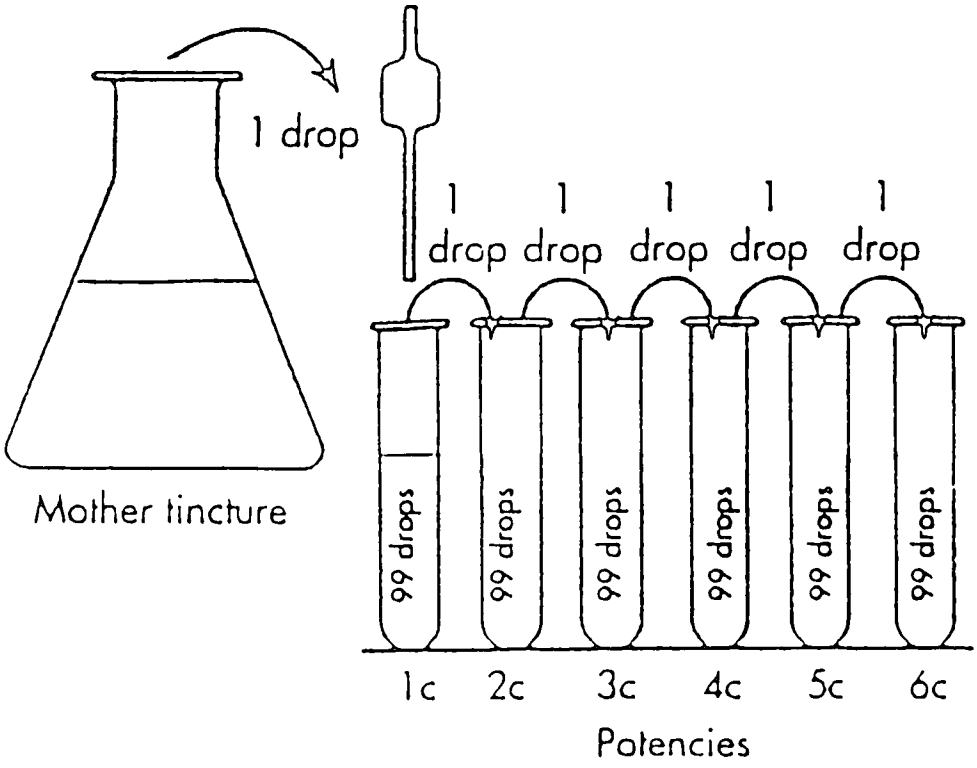

Figure 2.3 Schematic representation of the potentisation process (From Kayne 1997: 49)

The mother tinctures from which the dilutions are made are generally produced by extraction of the source material in water/alcohol mixtures (Kayne, 1997:47-48). However, where the base substances are insoluble, they are first triturated with lactose powder up to the 3cH level, after which they become soluble.

When this process was first developed by Samuel Hahnemann in the late 18th century his initial aim was to reduce the toxicity of medicines while still maintaining their therapeutic efficacy. In due course Hahnemann added succussion to the process of preparation of homoeopathic medicines, however, his initial reason for introducing succussion into the

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process is not given in any of his known writings. He began to use the term potentisation in 1827 (Koehler 1986:33) to refer to the process of dilution with succussion and he came to consider it to be an essential aspect of the preparation of homoeopathic medicines, an aspect whereby the healing potential of inert or toxic substances is released and refined (Hahnemann, 1996: 269).

2.9.3.1 The role of succussion

Succussion is considered an essential part of the preparation of homoeopathic medicines and it is considered to be the factor that distinguishes potentisation from simple or progressive dilution. Kayne states that, "This agitation is vitally important to the therapeutic efficacy of the remedy; dilution alone is not sufficient to produce the phenomenon." (1997:49). Vithoulkas (1980: 167) also maintains that "Succussion adds kinetic energy to the solution which is crucial ... We also know that the more there is succussion and dilution, the more the therapeutic power is increased, even beyond the point of there even being one molecule of the original substance remaining." However, Vithoulkas also acknowledges that there is as yet no available explanation for the phenomenon of potentisation. In the process of serial dilution and succussion Kayne (1997:50) acknowledges that for many people, especially for those educated in science, this concept is difficult to accept. Lessell (1994: 2) goes further in asking, "What science is it that knows neither what it dispenses, nor in which quantity." Hahnemann himself in his *Organon* gives the following quasi-scientific explanation of the phenomenon of potentisation, "It becomes evident that the material part (of an original substance) by means of such potentisation ... will ultimately dissolve into its individual spirit-like essence. In its crude state, therefore, it may be considered to consist really only of this undeveloped conceptual essence" (Hahnemann, 1996: aphorism 270). However, modern homoeopathic researchers theorise that the mechanism of action of homoeopathic high dilutions, i.e. those above 12cH, are as a result of biophysical and not biochemical interactions (Resch and Gutmann, 1991:191-213, Towsey and Hasan, 1995, Bellavite and Signorini, 1995: 243-301).

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Various theories have been proposed to explain the action of homoeopathic medicines in ultra high dilution. These theories have been classified into three groups by Lessel (1994:21); geometrical theories, dynamic field theories and submolecular theories. Several of these, which have special relevance to the role of succussion in the production of homoeopathic medicines are discussed below.

Most of the geometrical theories relate to the ability of liquid water to form irregular crystalloid globules from polymeric clusters, which clusters some assume to be the "messengers" that carry homoeopathic therapeutic information. It is proposed that during succussion each cluster is able to adopt an individual conformation that is characteristic of the solute. In addition, because of expected aggregation of water molecules by hydrogen bonding, each cluster is expected to enlarge while maintaining its unique crystalloid architecture, thus forming an encoded message of biological significance (Lessel, 1994:15-16) (see fig. 2.4).

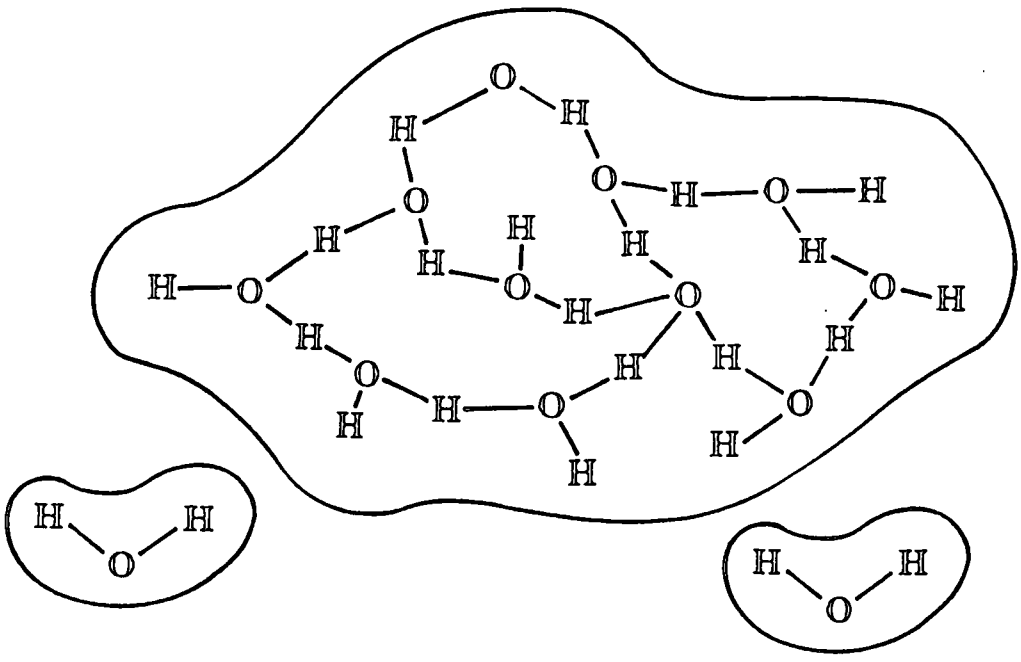

Figure 2.4 Schematic representation of the proposed structure of water clusters (From Lessel 1998:16)

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Anagnostatos (1994) proposed a theory based on the cluster hypothesis, in which clathrates, which are basically small water clusters are the means of information transfer in the biological system. Clathrates are crystalline structures that are formed when a solvent encases a solute in a hydrogen-bonded water shell-like lattice structure. As the mixture is succussed, the solute and clathrate separate and the clathrate maintains its essential shape, although it shrinks due to the absence of the hydrophobic forces between the solute and water molecules of the clathrate. The shrinking is said to increase the strength of hydrogen bonds and also results in the formation of a new (mantle) clathrate shell, similar in shape and size to the original (core) clathrate, by the layer of water molecules contiguous to the clathrate shell. Continued succussion leads to the separation of the core and mantle clathrates and repetition of the process leads to the formation of a multitude of clathrates, which are perpetuated beyond the point at which the original solute has been diluted out of the solution. These clathrates are proposed to be the means by which the biological "message" is maintained and transmitted beyond the point at which there are no molecules of the original substance remaining.

Another popular geometrical theory concerns the formation of hydration shells. Hydration shells are formed by the close electrostatic association of polar water molecules with ions or the poles of other polar molecules (see fig. 2.5 - overleaf).

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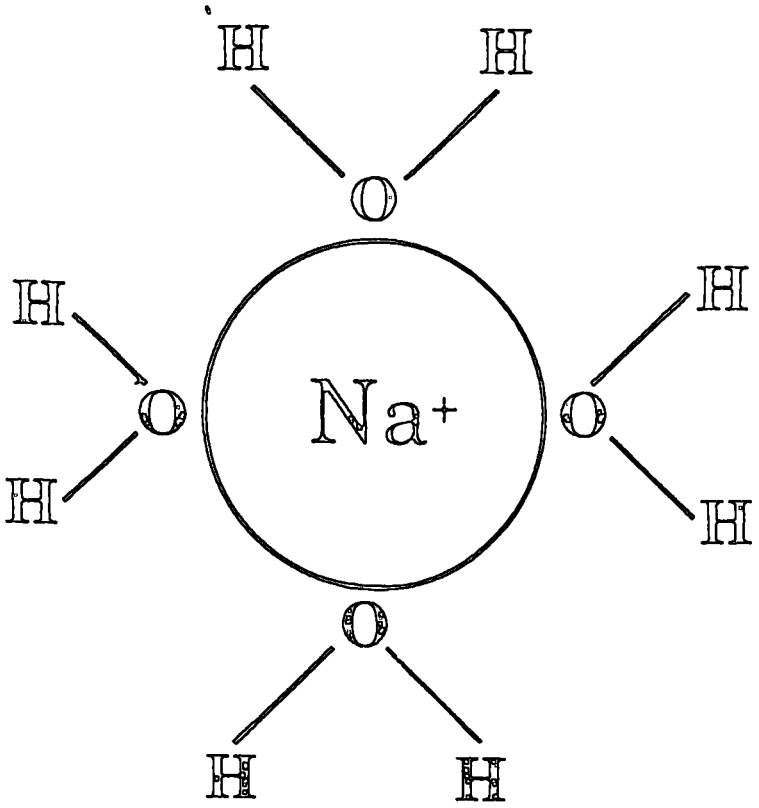

Figure 2.5 Schematic representation of proposed structure of hydration shells (from Lessel, 1998:18)

It is maintained that the hydration shell produces a negative shape, somewhat like a cast, of the engulfed ion or molecular pole (Lessel: 18-19). Explanations of potentisation based on this theory propose that during dilution the shell splits open to release the engulfed particle and then the fragments of the shell either reform or collapse down to form a smaller positive representation of the original particle shape, which representations are the carriers of the original "message".

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Towsey and Hassan (1995) propose that the layers of water molecules around the solute molecule are arranged and maintained in a semi-crystalline array, the shape of which is determined by that of the enclosed molecule – thus far their proposition is similar to the clathrate theory of Anagnostatos (1994). However, they maintain that when the remedy is succussed parts of these crystals break off and form "seeds" for new crystals, which have the same conformation as the original crystals, they now, however, exist independently of the solute molecules. As to how these crystals produce their biological effects they propose the following: That due to its unique crystalline structure each water envelope vibrates at its own unique frequency, and these crystals are able to absorb and emit radiation at specific frequencies. Furthermore, since studies of biological luminescence have suggested that a significant amount of cellular control is achieved by the absorption and emission of coherent radiation within cells, they propose that each potentised water crystal acts as a radiation transmitter and receiver in the bloodstream, which radiation is able to penetrate cells and activate or deactivate enzymes by means of resonance.

Of the dynamic field theories of the mechanism of potentisation, those proposed by Resch and Gutmann (1987,1991) enjoy the most support. All the dynamic field theories share some propositions. They maintain that the solute produces an electromagnetic field which has a characteristic vibrational frequency, they further propose that the solvent becomes "attuned to" or "resonates with" this frequency and that this characteristic vibrational frequency is maintained by the solvent even after the solute has been dissolved out of it.

Resch and Gutmann (1987, 1991) explain the role of dilution and succussion in the potentisation process in terms of a supermolecular system organisation of liquid water. They propose that when a so-called "mother tincture" is diluted in pure solvent, two similar systems come into contact, viz. the more differentiated solute with its better developed static structural aspects and the less differentiated and dynamically more active solvent. In the process of dilution, which involves mixing the two systems, a new system is formed which has a system organisation that differs from those of its components. The greater "openness"

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and the better-developed dynamic aspects of the pure liquid allow the integration of structural information from the mother tincture into the more diluted solution. As the concentration of the solute in water decreases, the solution shows greater similarity to pure water, but the information from the solute is not lost, it is rather spread over the whole of the more dilute solution, and thus the "message" is maintained and even amplified. Essentially the theory of Resch and Gutmann hinges on their proposal that the collective behaviour of a group of molecules cannot be accurately deduced from experimental observation of their individual properties, a proposition that Lessell recognises as an uncertainty principle of great significance (1994:33).

According to this theory, at each stage of attenuation the less differentiated system i.e. the pure solvent as the diluent, is modified by the structural aspects of the differentiated system i.e. the solution, to a greater extent than vice versa. In this way, integral structural information from the solution is spread over the new system, and this process is said to be facilitated by agitating the solution. Shaking the mixture adds energy and intensifies the "existential conflict" between the two systems of organisation. In the course of responding to this conflict, the energy is redistributed within the developing system organisation and the concentration of dissolved gas molecules is increased. Because the solute molecules of the mother tincture are subordinated to the dissolved gas molecules, the latter will consume a greater amount of the additional energy provided by the shaking process, and the additional transfer of structural information from the original solute molecule to the gas molecules is accomplished. In this way, the structural information originally present in the solute is readily integrated and dynamically maintained in the more dilute solution. Resch and Gutmann (1987:329-330), discuss the uptake of energy by a succussed solution, using the example of a sodium chloride solution. On heating solid NaCl a certain amount of thermo luminescence energy is released. If, however, a liquid solution of NaCl is succussed and then allowed to slowly crystallise by evaporation, the thermo luminescence energy released from these crystals is about twice as high as that of NaCl crystals obtained from an unsuccussed solution.

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Antonchenko and Ilyin (1992) maintain that dissipative structures are formed by water which has formed hydration shells, clathrates and similar microclusters. The stability of these microclusters depends on the movement of protons along spiral water molecule chains within these structures. They attest that the ability of various dissipative structures to remain stable in water systems is demonstrated by their presence in the earth's electromagnetic field, and by the stabilising processes of proton transfer along hydrogen-bonded chains in these structures. The act of succussion causes the formation of cavitation microbubbles in a liquid, as these bubbles collapse, dissociation of water molecules occurs which results in the release of protons which is necessary for the stabilisation of the dissipative structures.

The many theories that have been proposed to explain the working of homoeopathic potencies and the role of succussion in the production of these all are hampered by the same difficulty, i.e. the absence of experimental models and equipment sensitive enough to detect and measure the subtle changes in structure and effect that succussion is proposed to produce in homoeopathic potencies. The hope is that as more sensitive methods are developed the working of homoeopathic potencies and the role of succussion, if any, will be elucidated.

The process of potentization is also one aspect of homoeopathy that concerns many Christians, who consider succussion a ritual that invokes malignant spiritual forces. Bambridge (1998:7) states that, "Hahnemann himself was in no doubt that his techniques of dilution and succussion were a process of releasing an energy that he regarded as essentially immaterial and spiritual.... I believe that the process of dilution and particularly succussion can invoke the activity of spiritual forces whose intentions are not as benign as those of some homoeopaths." Samuel Pfeiffer (1988:72) says of homoeopathy that, "Its healing power is coming from cosmic power transferred to the remedy through the ritual of potentisation". This controversial area of homoeopathy was also investigated by this study, which aimed to determine if there are any statistically significant differences between the actions of ABA dilutions prepared by serial dilution and succussion, as opposed to those prepared by serial

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dilution alone.

2.9.4 Homoeopathic agricultural research

The investigation of the effects of homoeopathic medicines in the agricultural context is a relatively recent development. It has the advantage of being unencumbered by the various ethical considerations that exist in doing human or animal studies, as well as the fact that plants and seeds respond relatively quickly to applied stimuli, thus reducing the time needed for studies and making this research relatively economical.

Scofield (1984) discusses a variety of studies that have been conducted to test the efficacy of homoeopathic treatment in the agricultural context. He concludes that, despite many studies having been undertaken, little firm evidence emerged to demonstrate the efficacy of homoeopathic medicines - more often than not due to dubious experimental methods or unreliable statistical analyses being employed.

Studies relevant to this research are now discussed:

Betti *et al* (1994: 195-201) conducted a randomised laboratory trial where homoeopathic dilutions of *Arsenicum album* (Arsenic trioxide) were tested for their effect on seed germination. The percentage of germinated seeds as a function of time was calculated and compared to the Poisson distribution of the distilled water group, which allowed for parametric statistical evaluation of the significance of the different treatment groups. Three treatment groups showed a significance level of less than 1% and another was below 5%. The experimental results showed that differences between the treatment groups could not be explained as a mere effect of intrinsic seed variability. Average germination time statistically analysed by means of a one-factor analysis of variance did not yield significant results.

Bornoroni (1991) conducted experiments on fragments of oat seedlings (coleoptiles). He pre-

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treated half of the oat coleoptiles with dilutions of $CaCO_3$ in the 5cH, 15cH and 30cH potency levels, i.e. 10^{-10}, 10^{-30} and 10^{-60} respectively, and then ten hours later, cultured all of the coleoptiles in the presence of 100μM of IAA (the plant growth regulator indolacetic acid). The lengthening of the coleoptiles was taken as a measure of IAA activity and the synergistic action of $CaCO_3$. For comparison purposes one batch of segments were treated with homoeopathically diluted $CaCO_3$ without subsequent IAA treatment.

From 6 hours after being treated with the IAA, those specimens that had been pre-treated with the 5cH potency of $CaCO_3$, showed an 8-9 % growth increase when compared to those treated with IAA alone. Those pre-treated with the 15cH potency of $CaCO_3$ showed a statistically insignificant increase in growth compared to those treated only with IAA. And those specimens pre-treated with the 30cH potency of $CaCO_3$ showed a growth increase of 3-5% when compared to those treated only with IAA, which growth increase is not statistically significant. It was also found that when applied exclusively i.e. without IAA the 5cH, 15cH and 30cH potencies of $CaCO_3$ had no effect on the growth of coleoptile segments, and it was therefore concluded that $CaCO_3$ does not have an activity similar to that of auxin, but does appear to synergistically augment the action of the auxin IAA, at the 5cH potency level.

Hopkins (1998) investigated the biological effects of homoeopathic medicine treatments (Sulphur, Nitric acid and Camphor in 3cH, 9cH, 15cH and 30cH potencies) on lettuce seed germination. A germination index was calculated, and results were analysed by means of a multifactorial analysis of variance. He concluded that biological effects are evident, represented by statistically significant results between treatments. He recommends germinability studies as a possible methodology for testing the efficacy of homoeopathic medicines without the ethical implications involved in patient or animal based research. He also recommends that the effects of plant growth substances or plant hormones prepared according to homoeopathic principles be investigated. This study was conducted using a homoeopathically prepared plant hormone.

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Jones and Jenkins (1981) studied the effects of mechanical succussion on plant growth. They tested the action of 30 succussions of *Kalium carbonicum* (Potassium carbonate) in 13cH to 17cH on wheat coleoptile growth responses. After 30 days the potencies showed a loss of activity but, once resuccussed, values comparable to the initial effects were obtained.

Jones and Jenkins (1983) conducted a study on yeast cells using *Pulsatilla* in 4cH and 8cH potencies, to test the growth response to different numbers of succussions. They noted a gradual but significant increase in growth responses as the number of succussions increased up to 60 and thereafter a slight decrease was noted.

Christie (1995) undertook a study with the aim of investigating the relevance of succussion in the preparation of homoeopathic medicines. The homoeopathic medicines *Pulsatilla, Kalium carbonicum* (Potassium carbonate), *Natrum muriaticum* (Sodium chloride) and *Psorinum*, at 8cH potency, were used. Medicines were prepared firstly by hand succussion and then by mechanical succussion, using 0, 40, 60, 80, 100, 120, 140 and 160 succussions. Each medicine was added separately to a *Saccharomyces cerevisiae* culture and the growth of the yeast was then determined, using the direct cell counting method. Her findings indicated that succussion plays no role and that the relevance of succussion in the preparation of homoeopathic medicines is questionable. She recommends that in future research emphasis should be placed on developing a reliable and sensitive system for *in vitro* testing of homoeopathic medicines. The α-amylase system is such a reliable and sensitive system. In addition it also has the advantage of eliminating the subjectivity of patient based studies as well as being reproducible.

Steele (1999) conducted a study of the effects of homoeopathic dilutions of Gibberellic acid on the synthesis of α-amylase in barley endosperm half-seeds. He conducted 2 series of experiments. The first were serial dilutions of gibberellic acid prepared with succussion between each stage and the second series consisted of dilutions prepared without intervening succussion. He found that the ultra high dilutions of gibberellic acid were capable of causing

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significant α-amylase synthesis, but that there was no significant difference between the results of the remedies prepared with or without succussion. He concluded that succussion is not a significant factor in the efficacy of homoeopathic medicines. Among his recommendations were that other plant hormone substances be used in the same experimental model and that future homoeopathic trials utilise the unsuccussed dilutions as an additional control. This study aimed to use a similar experimental model, substituting abscisic acid (a germination inhibitor) for gibberellic acid (a germination promoter).

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CHAPTER THREE

RESEARCH MATERIALS AND METHODS

3.1 Introduction

The study was carried out in the Crop Science Laboratory of the School of Agricultural Science and Agribusiness, Faculty of Science and Agriculture at the University of Natal, Pietermaritzburg. See Figure 3.1 for a summary of the experimental protocol.

1. Prepare dilutions – succussed and unsuccussed.
2. Prepare α-amylase standard curve.
3. Prepare seed.
4. Prepare petri dishes with the different treatment solutions.

a. Incubate petri dishes in the dark in a growth chamber set at 15°C for 48 hours.

b. Terminate the germination process by placing the petri dishes in a freezer set at -20°C.

5. Enzyme extraction:

a. Thaw the petri dishes by leaving them at room temperature for 5 minutes.

b. Macerate the contents of each petri dish with 10ml of extraction buffer.

c. Decant into numbered centrifuge tubes.

d. Incubate the samples for 60 minutes in a shaker bath set at 30°C for 60 minutes.

e. Filter.

f. Make up to 10ml with the extraction buffer.

6. Enzyme assay:

a. Place samples in a shaker bath set at 50°C then add 1 Phadebas tablet per sample. Incubate for 10 minutes and then add 1ml NaOH to each sample to terminate the reaction.

b. Filter.

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c. Make up to 10ml with the extraction buffer.

d. Using a spectrophotometer, read the absorbance of each sample at 620nm. Record the readings in table form.

7. Analyse the data.

Figure 3.1 Summary of the experimental protocol

3.2 Study population

The study was conducted on barley seeds (*Hordeum vulgare* Stirling) of the 1999 harvest that were obtained from the Caledon Farmers Cooperative, Western Cape, South Africa. The seed was obtained directly from farmers rather than a commercial seed supplier to ensure that the seed had not been treated with any exogenous substances (Cairns, 2000). These seeds were cut in half transversely, retaining the endosperm-half for experimental purposes while discarding the embryo-half. A total of 1200 half-seeds were utilised.

3.3 Abscisic acid

This was in the form of cis,trans-ABA obtained from Saarchem-Holpro Analytic (Pty.) Ltd., Krugersdorp, South Africa. The cis,trans-ABA is soluble in ethanol and water and the mother tincture was prepared by dissolving 1mg of cis,trans ABA in 1ml of 99% alcohol and 1ml of distilled water.

3.3.1 Selection of homoeopathic dilutions of cis,trans-ABA

As the Avogadro limit is crossed by dilutions from 12cH onwards, solutions of 4cH, 9cH, 15cH, 30cH and 200cH were selected, representing dilution levels on either side of the Avogadro limit. This was to allow for distinctions to be made between biochemical and

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biophysical means of action. These dilution levels were also chosen because they are similar to those used by Hopkins (1998) and Steele (1999); thereby facilitating a greater degree of relevance to any similarities or differences noted between these experiments.

3.3.2 Preparation of homoeopathic dilutions of cis,trans-ABA

The homoeopathic dilutions of cis,trans-ABA were prepared by the researcher under laminar flow conditions at the laboratory of the Homoeopathic department of the Natal Technikon, Durban, according to the method specified in the German Homoeopathic Pharmacopoeia (GHP) (British Homoeopathic Association, 1985). Preparation was in accordance with Method 5a (British Homoeopathic Association, 1985:20-21). Although dilutions are usually made up in alcohol, in this case they were made up in distilled water, from the 1cH potency onwards, as alcohol may have an effect on plant growth regulator activity (Cairns, 2000). All glassware was sterilised by first rinsing in distilled water and then baking in an oven set at 180°C for three hours.

The stock solution was prepared by dissolving 1mg of cis,trans ABA in 1ml of 99% alcohol and then adding 1ml of distilled water. From this stock solution two sets of dilutions were made. For the first method (serial dilution with succussion) a set of dilutions was prepared, in distilled water, by serial dilution and succussion, succussing each serial dilution 10 times. This was done in accordance with method 5(a) of the German Homoeopathic Pharmacopoeia (British Homoeopathic Association, 1985:20-21). For the second method (serial dilution without succussion) a set of dilutions was then prepared, in distilled water, by serial dilution without intervening succussion.

The dilutions required for experimental purposes, i.e. the 4th, 9th, 15th, 30th and 200th were made up in 100ml volumes by mixing 1ml of the preceding dilution with 99ml of distilled water (Millipore). To each of these dilutions was added 1ml of the incubation buffer, $Ca(NO_3)_2$, resulting in a 20mM solution of $Ca(NO_3)_2$. The proportion of the incubation buffer

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remained constant throughout the study in order to maintain optimal conditions for the synthesis of α-amylase, as calcium has been found to play a role in the synthesis of active and stable α-amylase molecules (Bush *et al* 1991).

The control solutions were prepared by adding 1ml of $Ca(NO_3)_2$ to 100ml of distilled water, resulting in a 20mM solution of $Ca(NO_3)_2$.

3.4 Preparation of the α-amylase activity standard curve

The method used was that of Moore (1981:178-179), as adapted by Steele (1999: 40).

Pure α-amylase (from Boehringer Manheim GmbH, Germany, 1350 enzyme units/mg) was used, as well as Phadebas Amylase Test tablets (from Pharmacia Diagnostics AB, Uppsala, Sweden). An enzyme unit is defined as the amount of enzyme catalysing the hydrolysis of 1μm glucosidic linkage at 50°C (Cairns and de Villiers 1986).

The α-amylase standard curve used for this experiment is shown in figure 3.2 overleaf.

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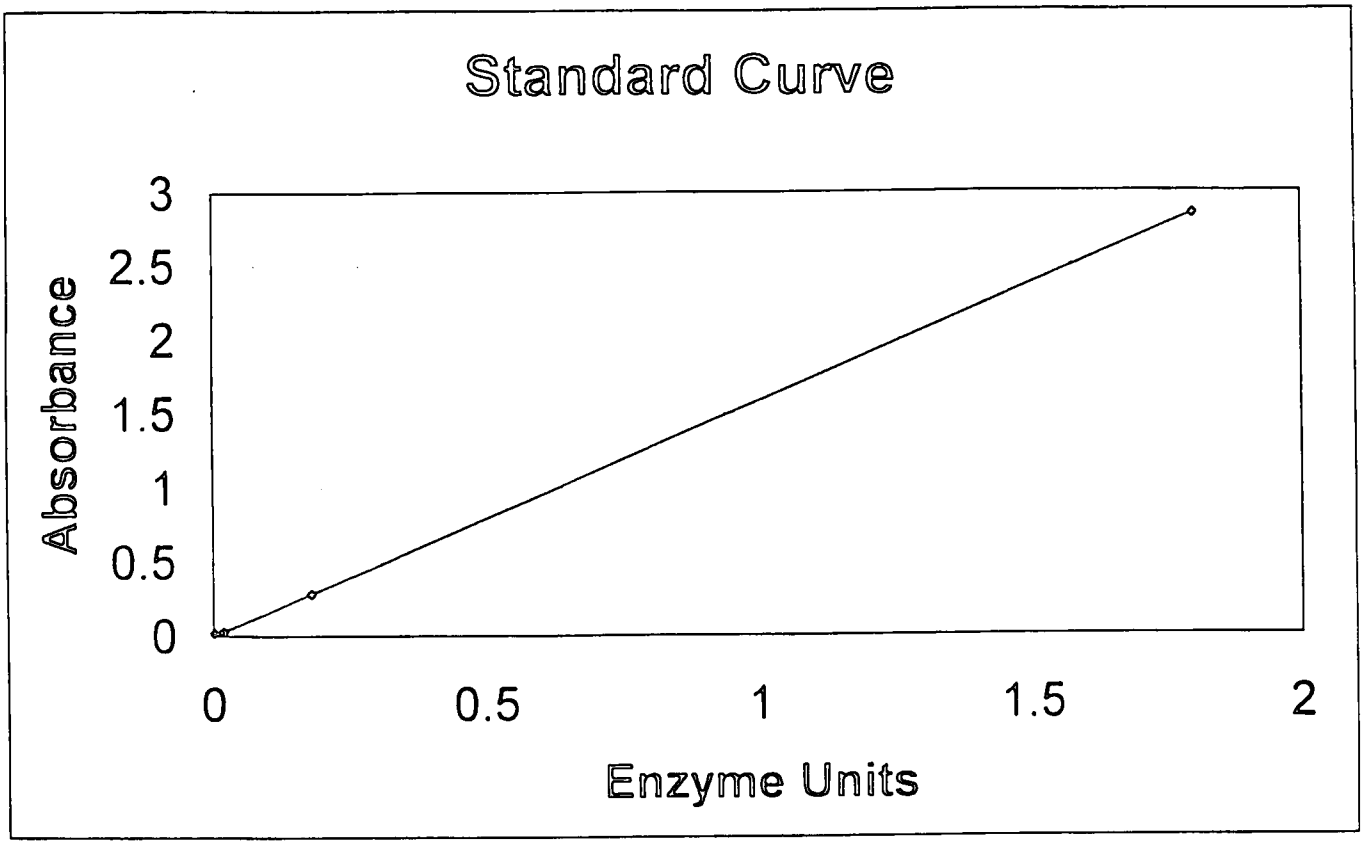

Figure 3.2 α-Amylase activity standard curve.

3.5 Preparation of the seed

The variety of barley seed used was *Hordeum vulgare* Stirling (untreated with any chemicals). 600 seeds were selected for each series, and in total 1200 seeds were used. Damaged or deformed seeds were excluded from selection. Each seed was cut in half transversely, the embryo-half was discarded and the endosperm-half was retained for experimental purposes. (see figure 2.2). In each series the endosperm half-seeds were divided into 30 groups of 20 half seeds each - 5 groups each for each of the 5 potency levels and 5 groups for the control.

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3.6 Preparation and treatment of the groups

The basic experimental design was that there were 2 series, succussed serial dilutions of ABA and unsuccussed serial dilutions of ABA. Each series consisted of 5 replications, and each replication consisted of 5 treatment groups. Each of the treatment groups were treated with a different homoeopathic dilution of ABA and the control group was treated with serial dilutions of the solvent i.e. distilled water made up to a 20mM solution of the incubation buffer i.e. $Ca(NO_3)_2$.

Each 9cm petri dish was lined with two 9cm No.1 Whatman filter papers and then each petri dish was labelled with the number of the series, the number of the repetition and the number of the treatment solution. The filter papers in each petri dish were moistened with 5ml of the relevant treatment solution, and then 20 half-seeds were placed into each petri dish, on top of the wet filter paper. The petri dishes were then each placed in a draw-string bag containing 2ml of distilled water to minimise evaporation and the bag was sealed and incubated for 48 hours in the dark at a constant temperature of 15°c.

3.7 Determination of α-amylase synthesis

3.7.1 Enzyme extraction

This methodology was based on that used by Cairns and de Villiers (1986) and Steele (1999). The same methodology was used for both succussed and unsuccussed dilutions.

After 48 hours, the incubation was terminated by placing the half-seeds in the freezer for 2 hours. Immediately prior to extraction, the contents of the petri dishes were thawed by leaving them at room temperature for five minutes. The half-seeds were then macerated with a mortar and pestle with 10ml of extraction buffer containing 58.4 mM (5.0g) sodium chloride and 1.14 mM (0.2g) calcium acetate. The slurry was then extracted in a shaker-bath

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for 60 minutes at 30°C, and then filtered through one thickness of Whatmann no. 1 filter paper into 30ml test tubes, which were made up to 10ml with the extraction buffer.

3.7.2 Enzyme assay

This methodology was based on studies by Cairns and de Villiers (1986) and Steele (1999). the same methodology was used for both succussed and unsuccussed dilutions.

The filtrate was incubated in the water bath for 10 minutes at 50°C. Dye-labelled substrate in the form of one Phadebas tablet was added to each test tube. This was done sequentially every minute, to ensure that each test tube had a full 10 minutes incubation. The tube was held on the mixer for 10 seconds to assist with dissolution of the tablet. After 10 minutes, the reaction was terminated by adding 1ml of 0.5 M NaOH (sequentially) to each test tube. Again the tube was held on the mixer for 10 seconds to assist with the mixing.

The homogenate was then filtered through one thickness of Whatmann no.1 filter paper and the resulting filtrate was made up to 10ml with the above-mentioned sodium chloride/calcium acetate buffer. The reference reading of the spectrophotometer was set on the "blank" sample, containing only the extraction buffer and then the absorbance of each sample was read at 620nm.

3.8 Data analysis

3.8.1 Calculation of enzyme units synthesised

α-Amylase enzyme activity is expressed as enzyme units per gram fresh weight of endosperm halves. An enzyme unit is defined as the amount of enzyme catalyzing the hydrolysis of 1m glucosidic linkage at 50°C (re: Phadebas tablets). By interpolation from the α-amylase standard curve, the α-amylase concentration corresponding to the absorbance value measured for each sample of incubated filtrate was calculated. This result was divided

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by the mean dry mass of the samples to arrive at the enzyme units synthesized by each sample.

3.8.2 Statistical methods

Statistical analysis was conducted using the univariate multifactorial Analysis of Variance (MANOVA) method.

There was only one dependent variable: reading.

This was a balanced multifactorial experimental design.

There were 2 methods.

There were 6 dilution levels per method.

There were 5 observations per dilution level.

3.8.2.1 Statistical model

The statistical model for the multifactorial ANOVA experiment is given as follows:

$Y_{ijk} = \mu + A_i + B_j + A_iB_j + \varepsilon_{ijk}$

Where:

μ is the overall or common effect;

A_i is the effect of the methods;

B_j is the effect of dilution levels;

A_iB_j is the interaction effect between methods and dilution levels;

ε_{ijk} are random error terms;

I = 1,2 = number of methods;

J = 1,……,6 = number of dilution levels per method;

K = 1,…..,5 = number of observations per dilution level.

The total number of rows in the data spreadsheet is equal to 2 x 6 x 5 = 60

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3.8.2.2 Procedure 1 : The univariate MANOVA to test main effects for significance

The effects of the two different methods i.e. succussed versus unsuccussed, and the dilution levels were tested for significance. In each case, the null hypothesis states that there is no significant difference between the categories of the main effects. The alternative hypothesis states that there is a significant difference between the two methods. The null hypothesis was rejected if the P-value was smaller than the level of significance, α, of the test. The null hypothesis was accepted if the P-value was equal to or greater than the level of significance, α, of the test. In this study, the level of significance, α, was fixed at the 0.05 level.

3.8.2.3 Statistical package

The statistical package GENSTAT was used for data entry and analysis.

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CHAPTER 4

RESULTS

4.1 The criteria governing the admissibility of results

The data utilised were optical density, from which the α-amylase enzyme units synthesised by each sample were calculated.

No data obtained was excluded from statistical analysis.

4.2 Physical Observations

There were no noticeable differences in appearance of the incubated endosperm halves between the control groups and all the treatment groups at any stage of the process.

4.3 Results

Table 4.1 shows the α-amylase enzyme units produced by the control as well as by the abscisic acid dilutions produced with intervening succussion. Figure 4.1 is a graphical representation of the mean results from table 4.1.

Table 4.2 shows the α-amylase enzyme units produced by the control as well as by the abscisic acid dilutions produced without intervening succussion. Figure 4.2 is a graphical representation of the mean results from table 4.2

Figure 4.3 is a graphical representation of the means of the two methods of preparation compared with the control.

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Table 4.1 Results: Method 1 – Dilution with succussion.

α-Amylase enzyme units synthesized per gram of dry barley endosperm half-seeds

Treatment	Replication						
	1	2	3	4	5	Mean	Median
Control	0.204	0.246	0.179	0.167	0.206	0.200	0.204
4CH	0.143	0.215	0.187	0.169	0.218	0.186	0.187
9CH	0.185	0.133	0.163	0.251	0.183	0.183	0.185
15CH	0.143	0.178	0.207	0.152	0.146	0.165	0.152
30CH	0.183	0.257	0.220	0.173	0.170	0.201	0.183
200CH	0.192	0.166	0.159	0.227	0.248	0.198	0.192

Table 4.2 Results: Method 2 – Dilution without succussion.

α-Amylase enzyme units synthesized per gram of dry barley endosperm half-seeds

Treatment	Replication						
	1	2	3	4	5	Mean	Median
Control	0.188	0.275	0.215	0.246	0.193	0.223	0.215
4CD	0.079	0.139	0.118	0.133	0.136	0.121	0.133
9CD	0.146	0.126	0.117	0.120	0.097	0.121	0.120
15CD	0.132	0.112	0.121	0.125	0.164	0.131	0.125
30CD	0.173	0.142	0.188	0.162	0.198	0.170	0.173
200CD	0.216	0.166	0.167	0.164	0.183	0.179	0.167

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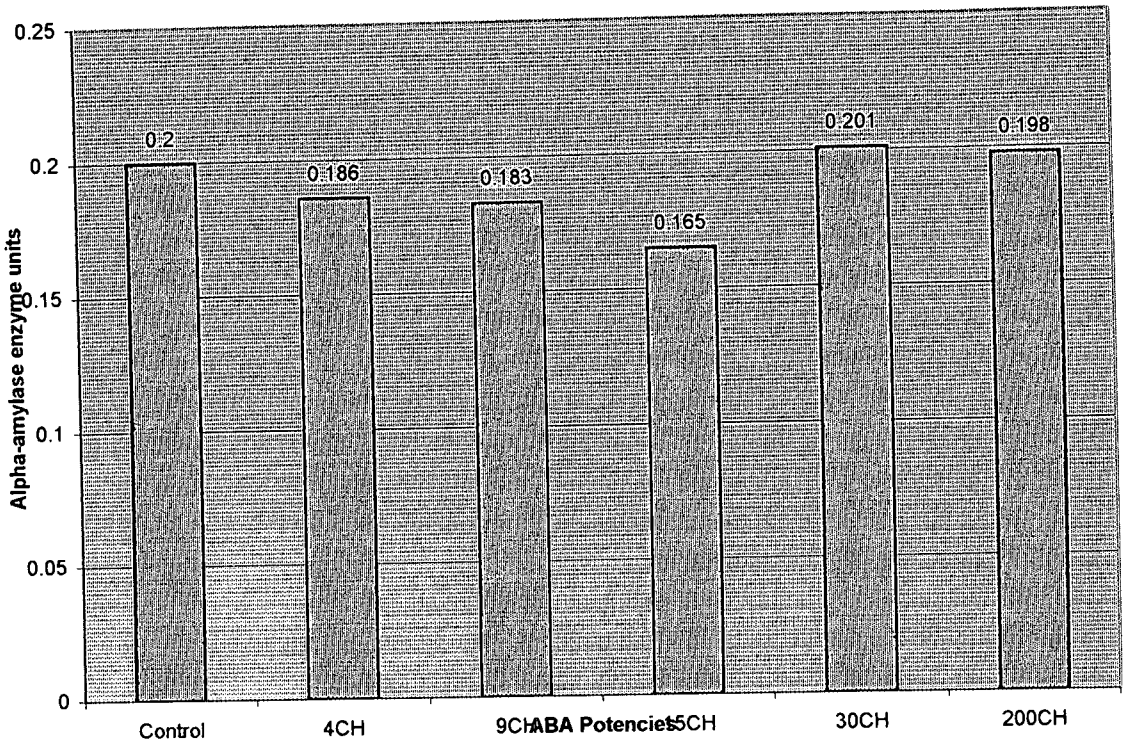

Figure 4.1 Method 1 – Effect of ABA dilutions produced with succussion on α-amylase synthesis in de-embryonated barley endosperm half-seeds

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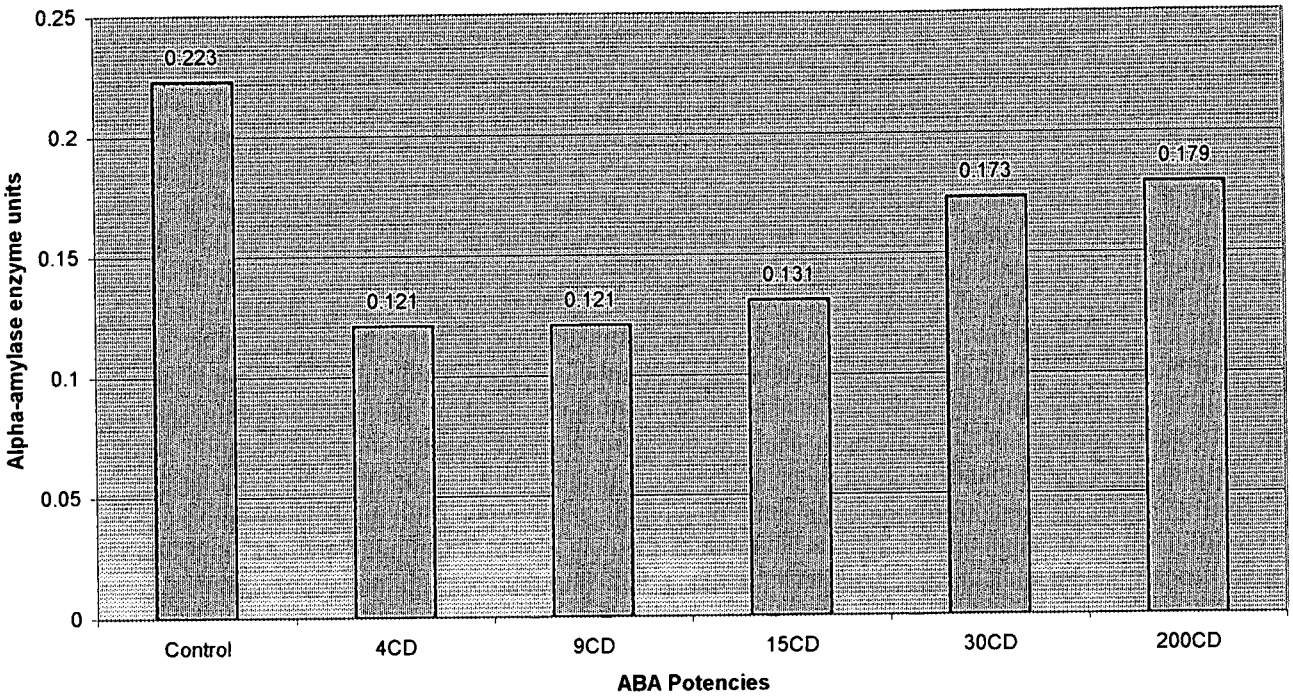

Figure 4.2 Method 2 – Effect of ABA dilutions produced without succussion on α-amylase synthesis in de-embryonated barley endosperm half-seeds.

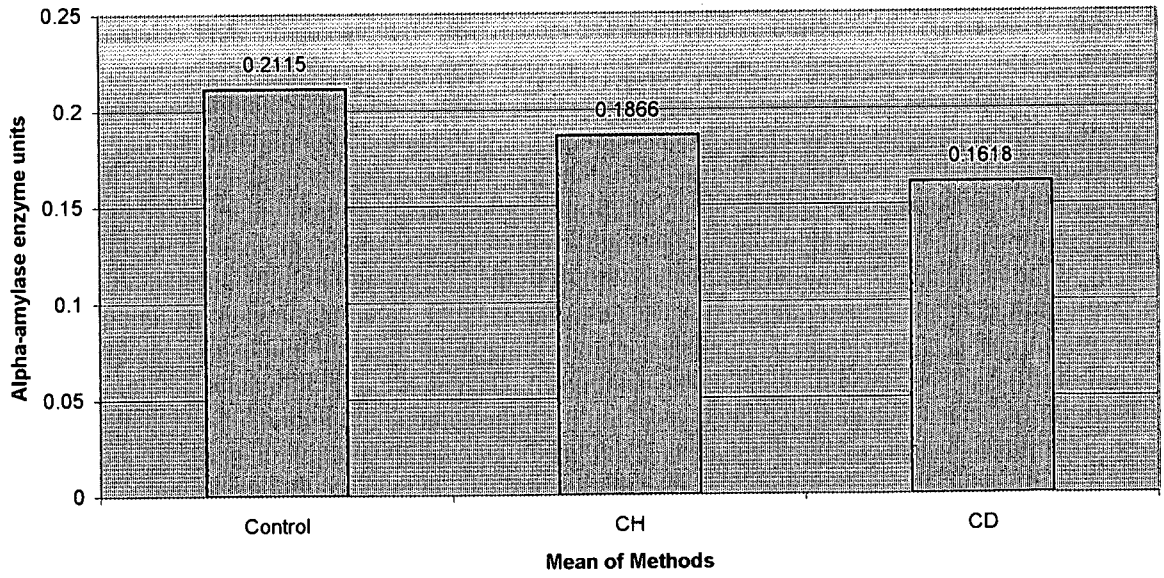

Figure 4.3 Comparison of the control with the means of the two preparation methods.

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4.3.1 Statistical analysis of results

The statistical analysis included a generalised ANOVA where concentration, preparation method and treatment were compared using the computer programme GENSTAT. A least significant difference (LSD) method was used to detect significant differences between treatment means at a 5% level of significance. (See Appendix 2)

Where,

H_o : null hypothesis

H_1 : alternative hypothesis

Decision rule:

At the $\alpha = 0.05$ level of significance,

1. Reject H_o if $p < \alpha$
2. Accept H_1 if $p > \alpha$

4.3.1.1Comparison of means

Table 4.3 shows the comparisons of the differences between the mean results of the different potency levels produced by dilution with succussion, with the least significant difference. The least significant difference is 0,04560 and differences greater than this, which indicate statistically significant differences, are denoted by an asterisk.

Figure 4.4 is a graphical representation of the results of table 4.3

Table 4.4 shows the comparisons of the differences between the mean results of the different potency levels produced by dilution without succussion, with the least significant difference. The least significant difference is 0.04560 and differences greater than this, which indicate statistically significant differences, are denoted by an asterisk.

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Figure 4.5 is a graphical representation of the results of table 4.4

Table 4.5 shows the comparisons of the differences between the mean results of the two methods of preparation and the control with the least significant difference. The least significant difference is 0.03224 and differences greater than this, which signify statistically significant differences, are denoted by an asterisk.

Figure 4.6 is a graphical representation of the results of table 4.5.

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Table 4.3 Differences between the ranked mean results of different potency levels produced by dilution with succussion, compared with the least significant difference. Statistically significant differences are denoted by an asterisk

Potency levels compared	Least significant difference	Difference between compared potency levels
9CH – Control	0.04560	0.0070
9CH – 30CH	0.04560	0.0120
9CH – 200CH	0.04560	0.0142
9CH – 4CH	0.04560	0.0262
9CH – 15CH	0.04560	0.0474 *
Control – 30CH	0.04560	0.0113
Control – 200CH	0.04560	0.0135
Control – 4CH	0.04560	0.0255
Control – 15CH	0.04560	0.0467 *
30CH – 200CH	0.04560	0.0022
30CH – 4CH	0.04560	0.0142
30CH – 15CH	0.04560	0.0354
200CH – 4CH	0.04560	0.0120
200CH – 15CH	0.04560	0.0332
4CH – 15CH	0.04560	0.0212

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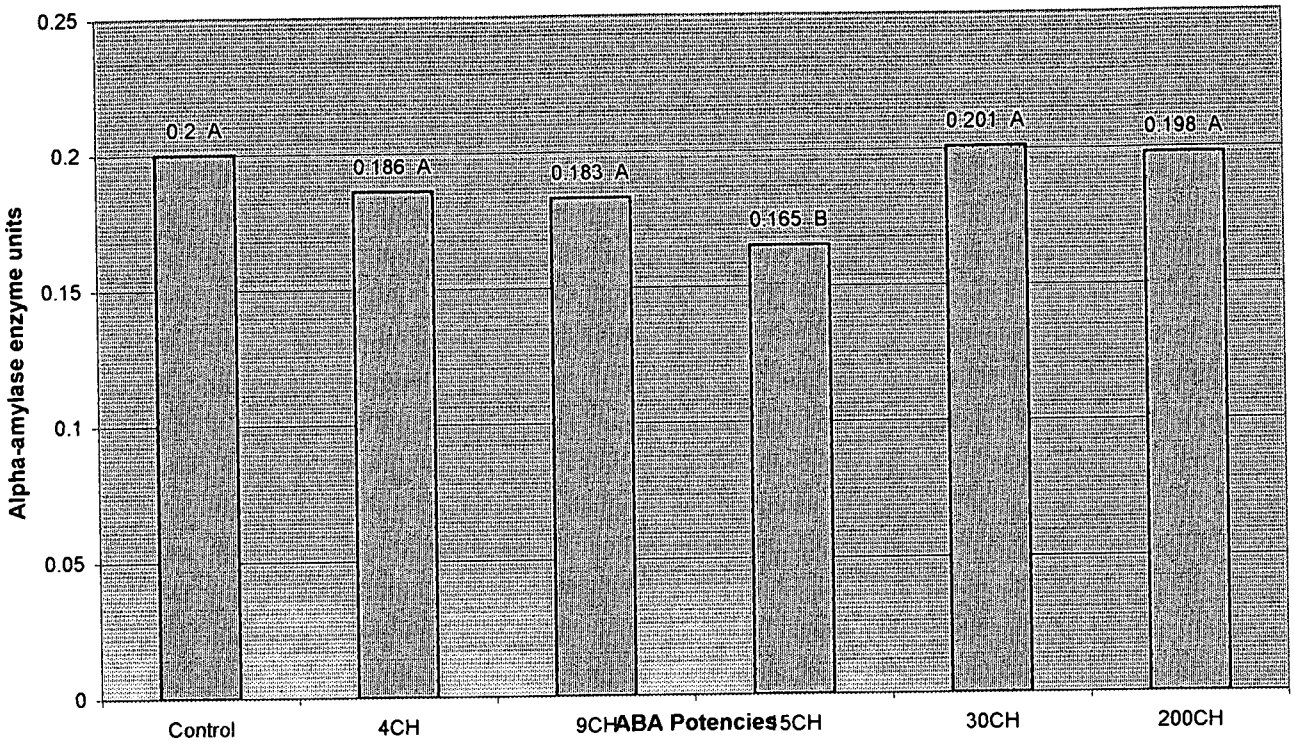

Figure 4.4 Comparison of mean results of different potency levels produced with succussion. Treatments denoted by the same letters are not significantly different at the 5% level.

It can be seen from figure 4.4 that the only potency level which differs significantly from the control and from the other potency levels is 15CH. This would appear to imply that the succussed potencies of abscisic acid were most inhibitory of α-amylase activity at the 15CH (10^{-30}) level. However, as this does not seem to be part of a trend exhibited by the decreasing concentration levels, it would be reasonable to ascribe this difference to chance.

The lack of significant differences between the potency levels themselves and between the potency levels and the control indicate that the succussed dilutions of abscisic acid had no significant effect on α-amylase synthesis.

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Table 4.4 Differences between the ranked mean results of different potency levels produced by dilution without succussion, compared with the least significant difference. Statistically significant differences are denoted by an asterisk

Potency levels compared	Least significant difference	Difference between compared potency levels
Control – 200CD	0.04560	0.0327
Control – 30CD	0.04560	0.0393
Control – 15CD	0.04560	0.0811 *
Control – 9CD	0.04560	0.0907 *
Control – 4CD	0.04560	0.0909 *
200CD – 30CD	0.04560	0.0066
200CD – 15CD	0.04560	0.0484 *
200CD – 9CD	0.04560	0.0580 *
200CD – 4CD	0.04560	0.0582 *
30CD – 15CD	0.04560	0.0418
30CD – 9CD	0.04560	0.0514 *
30CD – 4CD	0.04560	0.0516 *
15CD – 9CD	0.04560	0.0096
15CD – 4CD	0.04560	0.0098
9CD – 4CD	0.04560	0.0002

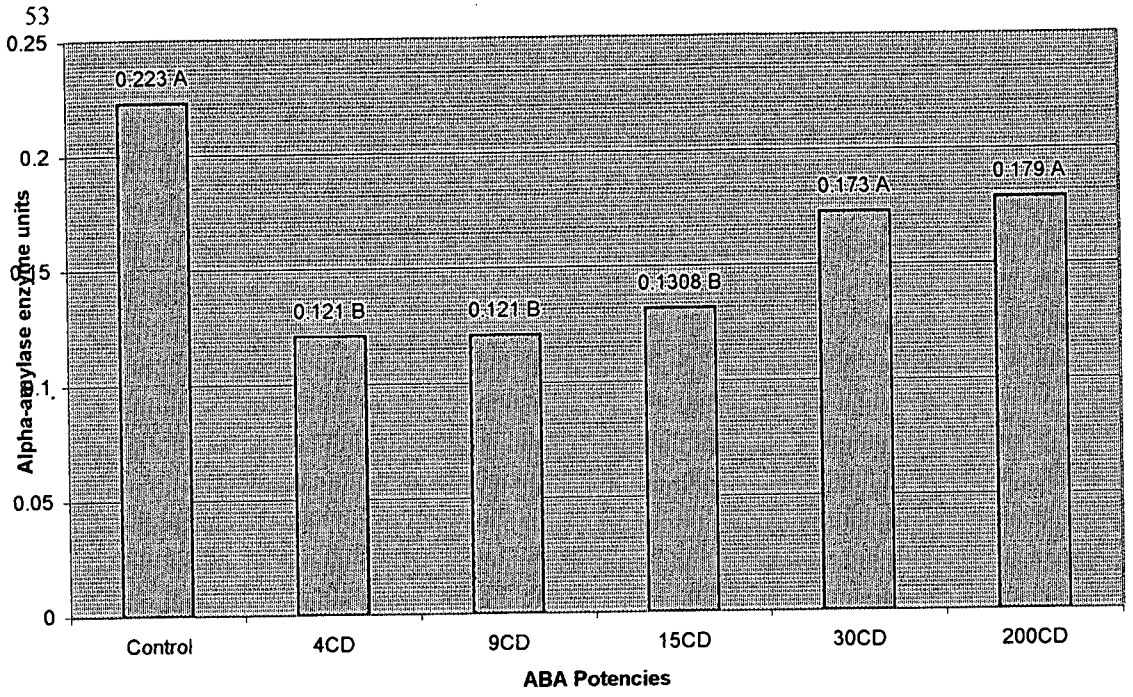

Figure 4.5 Comparison of mean results of different potency levels produced without succussion. Treatments denoted by the same letters are not significantly different at the 5% level.

It can be seen from figure 4.5 that significant differences between unsuccussed potency levels and the control occurred at the 4CD, 9CD and 15CD levels. The 30CD and 200CD levels were not significantly different from the control. These findings indicate that the inhibitory action of abscisic acid on α-amylase synthesis was maintained at the 4CD (10^{-8}), 9CD (10^{-18}) and 15CD (10^{-30}) potency levels. The finding of no significant differences between the 30CD and 200CD potency levels and the control, indicate that at these potency levels the inhibitory action of abscisic acid on α-amylase synthesis is not present.

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Table 4.5 Differences between the ranked mean results of the two methods of preparation, the control and the least significant difference. Statistically significant differences are denoted by an asterisk.

Methods compared	Least significant difference	Difference between compared methods
Control – CH	0.03224	0.0193
Control – CD	0.03224	0.0669 *
CH – CD	0.03224	0.0476 *

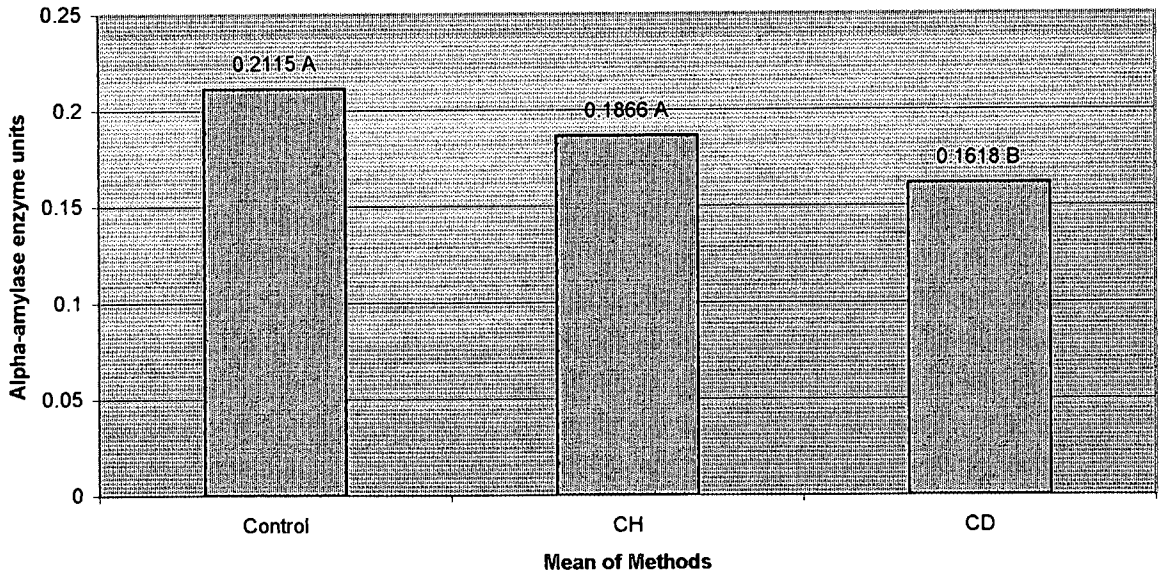

Figure 4.6 Comparison of the ranked mean results of the two methods of preparation and the control. Treatments denoted by the same letters are not significantly different at the 5% level.

Figure 4.6 demonstrates that there is no statistically significant difference between the mean of the controls and the mean of all the succussed dilutions, while there are statistically significant differences between the mean of the controls and the mean of all the unsuccussed dilutions, as well

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as between the means of the succussed and unsuccussed dilutions.

These findings demonstrate that the succussed dilutions of abscisic acid had no significant effect on α-amylase synthesis, while unsuccussed dilutions significantly reduced the synthesis of α-amylase. In fact, when compared with equivalent unsuccussed dilutions of abscisic acid, the process of succussion appears to have reduced the ability of the abscisic acid to inhibit α-amylase synthesis.

Table 4.6 Analysis of Variance table

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Control	1	0.015480	0.015480	12.03	0.001
Control.Prep	1	0.028417	0.028417	22.07	< .001
Control.Conc	4	0.013811	0.003453	2.68	0.042
Control.Prep.Conc	4	0.009001	0.002250	1.75	0.155
Residual	49	0.063078	0.001287		
Total	59	0.129787			

The analysis of variance table (table 4.6) shows the following:

1) That a significant difference exists between the results of the control and the mean of the two methods of preparation combined. The difference is highly significant: $p = 0.001$. Therefore, as $p < \alpha$, the null hypothesis, which states that no difference exists between the control and the test methods, is rejected. The conclusion therefore is that a highly significant difference exists between the control and the average of the two test methods, at the $\alpha = 5\%$ level. However, when considered individually, as demonstrated in figure 4.6, it is evident that only the unsuccussed dilutions were significantly different to the control, and that this difference contributed to the significant difference between the means of the two methods of

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preparation and the control. This means that the first hypothesis (see 1.4.1) is accepted, viz. that the succussed dilutions of ABA will have no effect on the synthesis of α-amylase in barley endosperm half-seeds; and, however, that the second hypotheses (see 1.4.2) is false, viz. that the unsuccussed dilutions of ABA will have no effect on the production of α-amylase in barley endosperm half-seeds.

2) That a significant difference exists between the two methods of preparation: $p < 0.001$, therefore, as $p < \alpha$, the null hypothesis (see 1.4.4), which states that no difference exists between the different methods of dilution on the production of α-amylase in barley endosperm half-seeds, is rejected. The results further demonstrate that the unsuccussed dilutions of ABA exhibited an inhibitory effect on α-amylase production, when compared with the control, but that the succussed dilutions of ABA were found to produce no effects significantly different to the control.

3) That no significant difference exists between the dilution levels: $p = 0.042$, therefore, as $p > \alpha$, the null hypothesis (see 1.4.4) is accepted, i.e. that there is no difference between the dilution levels on the production of α-amylase in barley endosperm half-seeds.

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CHAPTER 5

DISCUSSION OF THE RESULTS

5.1 Introduction

This study utilised dilutions of ABA ranging from 4CH to 200CH. If half-seeds treated with dilutions of ABA were found to synthesize α-amylase, this was considered objective evidence of the efficacy of isopathic treatment in this system. Furthermore, if α-amylase synthesis occurred in half-seeds treated with ABA dilutions higher than 12cH, this was considered objective evidence of the active nature of high homoeopathic dilutions. In addition, if there was found to be a significant difference between the efficacy of dilutions of ABA prepared by serial dilution and succussion, as opposed to those prepared by serial dilution alone, this was considered objective evidence that succussion plays a role in the production of homoeopathic medicines.

With reference to the above, the results of this study showed that high and ultra-high dilutions of ABA (up to 10^{-400}) are biologically active, as statistical analysis (ANOVA) indicated that there was a significant difference between the control group and the mean of the two treatment groups. Statistical analysis also showed that there was a significant difference between the two methods of dilution in terms of the biological activity produced. It was found that succussed dilutions of abscisic acid had no significantly different effect on α-amylase synthesis than the control substance, while the unsuccussed serial dilutions of abscisic acid did produce a significant reduction in the synthesis of α-amylase.

5.2 The Isopathic Principle

One of the aims of this study was to investigate the isopathic principle, which maintains that a substance, which in material doses has an inhibitory effect, will, in homoeopathic dilution, have a stimulatory effect (Gaier, 1991:290). According to this principle it would be expected that ABA, a

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germination inhibitor, would, in homoeopathic dilutions, stimulate germination and hence the production of α-amylase, more than the control substance. This was not shown as the amount of α-amylase produced by the ABA dilutions was consistently lower than that produced by the control. Therefore the isopathic principle was not demonstrated.

5.3 Biological activity of ultra-high dilutions of ABA

Although the isopathic principle was not demonstrated, what is of interest is that there was a significant difference between one of the treatment groups, the unsuccussed dilutions of ABA and the control, as this produced significantly less α-amylase than the control. This indicates that the inhibitory effect of ABA persisted in the unsuccussed dilutions of ABA.

Of particular interest is that this inhibitory effect was present up to the 15CD potency level, i.e. 10^{-30}, in the unsuccussed dilutions. This is in dilution far in excess of Avogadro's dilution limit, 6.022 x 10^{-23}, which is crossed at the 12CD dilution, beyond which theoretically not one molecule of the original substance remains. These results corroborate the results of Steele (1999), which showed that GA_3, when prepared in high and ultra-high dilutions, maintained the ability to stimulate α-amylase production, even up to the 200CH level, although, in this experiment the succussed dilutions did not demonstrate this biological activity. Giberrellic acid is a germination promoter and abscisic acid is a germination inhibitor; Steele found that the germination promoting effects of GA_3 were still present at dilutions in which there were theoretically none of the GA_3 molecules present, and the present study has found that the germination inhibiting effects of ABA were still present at dilutions at which there were theoretically no ABA molecules present.

This is of particular significance as these results demonstrate that the effects of the serial dilutions of ABA investigated are due to a factor intrinsic to the dilutions and not to one extrinsic to them such as suggestion or the placebo effect. This demonstrates that ultra-high dilutions can maintain a biological activity, an activity which cannot be accounted for by current biochemical theories.

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5.4 The role of succussion

It is generally accepted that an essential element in the preparation of homoeopathic medicines is the process of succussion. Kayne states that, "This agitation is vitally important to the therapeutic efficacy of the remedy; dilution alone is not sufficient to produce the phenomenon." (1997:49), and Vithoulkas (1980:167) also maintains that "Succussion adds kinetic energy to the solution which is crucial ... We also know that the more there is succussion and dilution, the more the therapeutic power is increased, even beyond the point of there even being one molecule of the original substance remaining." Studies by Christie (1995) and Steele (1999) both questioned the role of succussion, as they found no significant difference between the action of remedies prepared by dilution with intervening succussion and those produced by dilution without succussion. This study aimed to reinvestigate this area and the results demonstrated a statistically significant difference between the two methods of preparation (see Fig. 4.6).

It was found that the succussed potencies of ABA produced no significant difference in α-amylase synthesis when compared to the control dilutions, whereas the unsuccussed potencies caused significant reductions in α-amylase synthesis. The obvious conclusion is that the process of succussion interfered with the inhibitory stimulus of the ABA on α-amylase synthesis, or that the succussion rendered the ABA potencies biologically inactive.

An attempt could also be made to explain this phenomenon with reference to the isopathic theory mentioned earlier. As ABA is a germination inhibitor, isopathic theory would propose that succussed dilutions of ABA would stimulate germination. Compared to the effect of the unsuccussed ABA this appears to be what happened. It could be proposed that the suppressive effect of ABA on α-amylase synthesis, which was maintained at even very high dilutions, was modulated and became inhibited by the process of succussion i.e. the inhibitory effect of ABA was inhibited by the succussion. However, the inhibition of the suppressive effects of the ABA was not great enough to produce more α-amylase than was produced by the control substance. The modulation of the suppressive effects

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was not great enough to produce stimulation of α-amylase synthesis.

Although this study found that neither succussed nor unsuccussed dilutions of ABA produced more α-amylase than the control substance, it did demonstrate a significant difference between the effects of dilutions produced with intervening succussion and those produced without succussion, indicating that succussion is not a neutral act, but one which does generate a difference in the biological activity of the substance.

5.5 Homoeopathic dilutions and chemical theory

What is also of significance is that differences between the unsuccussed dilutions of ABA and the control were found at dilution levels up to 15CH(10^{-30}) potency level. This challenges a fundamental principle of chemistry, Avogadro's constant, which maintains that at dilution levels in excess of 6.022×10^{-23} no molecules of the original solute will remain (Gaier, 1991:47-48), and hence the biological activity of the solute will be absent. It is expected that as the solute molecules are diluted out by serial dilution and approach Avogadro's limit, chance may find one of the solute molecules being drawn up and included in the next dilution level. However, an attempt to explain the activity of the ABA dilutions on this basis can be dismissed for several reasons: firstly, chemical theory proposes that the activity of a substance is proportional to the concentration of that substance present, and if one or two molecules made it through the "net" these would be unlikely to have the significant effect that was noted from the dilutions tested. More compellingly, for each dilution level five replications were carried out and the chance of sufficient, if any, molecules slipping through the "net" to have significant biological activity in all five replications is highly unlikely. But, at each dilution level significant activity was produced by each of the five replications.

5.6 Homoeopathy and biophysics

The above would imply that an explanation of the effects of ultra-high dilutions based on the action of solute molecules alone is highly unlikely to be satisfactory. It is for this reason that many

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homoeopathic researchers have turned their attention to investigating possible biophysical explanations for the action of homoeopathic medicines, as discussed at length above (see 2.9.4).

These biophysical hypotheses of the action of homoeopathic medicines all seek to explain how these medicines can be active when none of the solute molecules remain. Most of the biophysical theories focus on conformational changes that are brought about in the structure of the solvent molecules by the process of dilution and succussion. Hence, these theories focus more on the solvent as the "message carrier" than on the solute, proposing that exposure to the solute, in the presence of succussion, causes an alteration in the conformation of solvent molecules such that they are able to carry information with biological activity. Whether the activity of the dilutions can be explained by theories based on clathrates, clusters, hydration shells or the induction of a dynamic field by the process of succussion, is beyond the field of this study; however, the prospect of instrumentation being developed that is sensitive enough to measure these very fine structural and dynamic changes is one to be looked forward to.

In contrast to the studies of Christie (1995) and Steele (1999), this study found significant differences between the two methods employed, which implies that there is a differing mechanism of action. As both methods underwent serial dilution and the only variable factor was the presence or absence of succussion, this must be the relevant factor. However, while the unsuccussed dilutions of ABA produced effects significantly different to the control, the results produced by the succussed dilutions of ABA, although significantly different to the unsuccussed dilutions, were not significantly different to the control. This does raise the question whether the act of succussion, in this experiment, eliminated any significant biological activity that the dilutions may have had. However, the fact remains that the succussed dilutions were significantly different in the results they produced to the unsuccussed dilutions. Why these differences arose and what their significance is are interesting questions that are beyond the scope of this study.

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5.7 Economical implications of the results

If further studies found that ultra high dilutions of Abscisic acid were actively able to inhibit germination in field trials, this could have potential economic implications, especially in the prevention of pre-harvest sprouting of wheat, which costs the agricultural sector dearly in lost revenue annually. In addition, studies have shown that ABA plays a role in the control of stomatal opening (Roberts and Hooley, 1988:169), and if ultra high dilutions of ABA are able to regulate stomatal opening they may have a role to play in agriculture by; reducing plant water consumption, reducing injury due to chilling and pollutant uptake, and even manipulating the senescence of plants. These possibilities all hold significant economical implications.

At present the employment of plant growth regulators in general is a growing trend and is expected to increase its share of the agricultural market. However the production and extraction of these plant growth regulator substances is costly, which renders their wide use uneconomical for the moment. If, however, these compounds were found to be effective in ultra high dilutions, then the economic viability of using them may become significantly more attractive to those in the agricultural sector.

5.8 Summary

The results of this study are ambiguous in that, although they found a significant difference between the actions of succussed and unsuccussed dilutions, there was no difference found between the actions of the succussed dilutions and the control, which implies that the succussed dilutions had no biological effect. One proposition to consider is that the process of succussion may have reduced the inhibitory effects of ABA on α-amylase synthesis, such that the succussed dilutions resulted in the synthesis of greater amounts of α-amylase than the unsuccussed dilutions. However, this speculative conclusion will need to be tested in other systems using succussed and unsuccussed dilutions of substances which have inhibitory actions on the system in material doses, in order to determine if this pattern is repeated in various systems. However, the fact remains that a significant difference was noted between the actions of succussed and unsuccussed dilutions of ABA, from which arises

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the conclusion that succussion is not a neutral process, but does have some effect on the biological activity of dilutions, albeit, as appears to be the case in this experiment, to render them biologically inactive.

Furthermore, this study, as many before it, challenges the limits for biological activity of ultra high dilutions set by Avogadro's constant of 6.022×10^{-23}, and proves that dilutions greater than this are able to be biologically active; however, the challenge remains as to discover precisely how this activity is produced.

This study also brings into question the theory of isopathy as the isopathic principle was not demonstrated.

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CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The results of this study into the effects of ultra high dilutions of ABA on the synthesis of α-amylase in barley endosperm half-seeds show that unsuccussed ultra high dilutions of ABA are biologically active. Furthermore they show a significant difference between the effects of succussed and unsuccussed dilutions of ABA. The unsuccussed dilutions produced less α-amylase than the control dilutions and the succussed dilutions, which indicates that the inhibitory effect of ABA was still present in dilutions produced by this method. Statistically significant differences were noted between treatment groups and control groups, as well as between the two methodologies employed, but not between the different dilution levels tested.

This study has further built on the study by Steele (1999) in demonstrating the feasibility and value of the barley endosperm half-seed model as an attractive experimental model for examining the action of ultra high dilutions of ABA, as it has shown itself to be both a sensitive and a specific test for the action of the ultra high dilutions of ABA and GA_3.

The results of this study bring into question the role of succussion in the preparation of homoeopathic medicines. The dilutions produced with succussion were found to not be significantly different in their effects to the control substance, while those dilutions produced without succussion were found to be significantly different to the control in the results they produced. While not explaining how these medicines produce their effects, this study does add impetus to the need for more research to be conducted into understanding the mechanism of action of homoeopathic medicines and, specifically, what role, if any, succussion plays in the production of homoeopathic medicines.

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6.2 Recommendations

1. That future homoeopathic trials, whether clinical, *in vitro* or *in vivo*, include a comparison of the effects of succussed and unsuccussed dilutions, as this study examined the difference between homoeopathic preparations produced with and without intervening succussion.
2. That further studies be conducted with other substances that have inhibitory actions on biological systems, in order to determine what differences, if any, are produced by using succussed and unsuccussed dilutions of the inhibiting substance in the system.
3. That this study be repeated in other laboratories in order to determine the reproducibility of the results.
4. That germination studies be conducted with whole barley seeds.
5. That a wider gradation of dilution levels be employed so as to illuminate any trends that may be present, as the wide gaps between dilution levels used in this experiment do not allow for an accurate trends assessment.
6. That various methods of preparing homoeopathic medicines be tested using this model, e.g. Korsakovian potencies and supersuccussed potencies.
7. That various external forces such as the heating of remedy dilutions, the exposure of remedies to substances such as Camphor and their exposure to sunlight be investigated using this model.
8. That a range of succussions be compared, e.g. 10, 100 and 1000, using this model. This would relate this research to that of Christie (1995) in which the effects of various numbers of succussions was evaluated.
9. That the effects of different degrees of dilution e.g. decimal (1:9), centesimal (1:99) and quinquagenimillesimal (1:49 999) be compared using this model.
10. That the study be repeated, using the same set of dilutions, over increasing time intervals e.g. 1 month, 6 months, 1 year, 5 years, etc. to test the longevity of the activity of the remedy dilutions.

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11. That the initial incubation period be varied.
12. That the initial incubation temperature be varied.
13. Use other seeds (e.g. wild oat) in the same experimental model.
14. Develop other *in vitro* models of enzyme modulation under the influence of ultra high dilutions, such as mammalian tissue models.
15. Investigate methods other than the Phadebas method for detecting the presence of α-amylase in the barley endosperm half-seeds.

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APPENDIX 1

The Results

Results of Abscisic acid study

1. Endosperm mass (in grams)

Treatment	Replication				
	1	2	3	4	5
Control	0.514	0.489	0.533	0.561	0.561
4CH	0.524	0.535	0.497	0.506	0.488
9CH	0.525	0.537	0.527	0.469	0.512
15CH	0.477	0.487	0.501	0.470	0.494
30CH	0.492	0.504	0.508	0.502	0.496
200CH	0.531	0.489	0.522	0.489	0.494

Method 1 – Dilution with succussion

Treatment	Replication				
	1	2	3	4	5
Control	0.501	0.529	0.513	0.520	0.533
4CD	0.537	0.506	0.495	0.516	0.523
9CD	0.532	0.553	0.539	0.534	0.524
15CD	0.564	0.555	0.523	0.541	0.534
30CD	0.538	0.518	0.544	0.559	0.540
200CD	0.525	0.599	0.550	0.535	0.517

Method 2 – Dilution without succussion

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Phadebas OD $^{(620)}$ reading

Treatment	Replication						
	1	2	3	4	5	Mean	Median
Control	0.173	0.198	0.157	0.154	0.191	0.175	0.173
4CH	0.123	0.190	0.153	0.141	0.175	0.156	0.153
9CH	0.160	0.118	0.142	0.194	0.279	0.179	0.160
15CH	0.112	0.143	0.171	0.118	0.119	0.132	0.119
30CH	0.148	0.213	0.184	0.143	0.139	0.165	0.148
200CH	0.168	0.134	0.137	0.183	0.202	0.165	0.168

Method 1 - Dilution with succussion

Treatment	Replication						
	1	2	3	4	5	Mean	Median
Control	0.155	0.240	0.182	0.211	0.170	0.192	0.182
4CD	0.070	0. 116	0.096	0.113	0.117	0.102	0.113
9CD	0.128	0.115	0.104	0.105	0.084	0.107	0.105
15CD	0.132	0.112	0.121	0.125	0.164	0.131	0.125
30CD	0.280	0.121	0.168	0.149	0.176	0.179	0.168
200CD	0.187	0.164	0.152	0.145	0.156	0.161	0.156

Method 2 - Dilution without succussion

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APPENDIX 2

STATISTICAL ANALYSIS OF THE RESULTS

***** Analysis of variance *****

Variate: adsorb

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Control	1	0.015480	0.015480	12.03	0.001
Control.Prep	1	0.028417	0.028417	22.07	<.001
Control.Conc	4	0.013811	0.003453	2.68	0.042
Control.Prep.Conc	4	0.009001	0.002250	1.75	0.155
Residual	49	0.063078	0.001287		
Total	59	0.129787			

***** Tables of means *****

Variate: adsorb [Grand mean 0.1760]

Control	1	2
	0.2119	0.1688
rep.	10	50

Control	Prep	Control	CH	CD
1		0.2119		
	rep.	10		
2			0.1926	0.1450
	rep.		25	25

Control	Conc	0.00	4.00	9.00	15.00	30.00	200.00
1		0.2119					
2			0.1537	0.1669	0.1480	0.1866	0.1888

Control	Prep	Conc	0.00	4.00	9.00	15.00	30.00	200,00
1	Control		0.2119					
		rep.	10					
2	CH			0.1864	0.2126	0.1652	0.2006	0.1984
		rep.		5	5	5	5	5
	CD			0.1210	0.1212	0.1308	0.1726	0.1792
		rep.		5	5	5	5	5

*** Standard errors of differences of means ***

Table	Control	Control Prep	Control Conc	Control Prep Conc	
rep.	unequal	unequal	10	unequal	
d.f.	49	49	49	49	
s.e.d.		0.01605X		0.02269	min.rep
	0.01243	0.01342	0.01605	0.01965	max-min
		0.01015		0.01605X	max.rep

(No comparisons in categories where s.e.d. marked with an X)

*** Least significant differences of means (5% level) ***

Table	Control	Control Prep	Control Conc	Control Prep Conc	
rep.	unequal	unequal	10	unequal	
d.f.	49	49	49	49	
l.s.d.		0.03224X		0.04560	min.rep
	0.02498	0.02698	0.03224	0.03949	max-min
		0.02039		0.03224X	max.rep

(No comparisons in categories where s.e.d. marked with an X)

*** Least significant differences of means (1% level) ***

Table	Control	Control Prep	Control Conc	Control Prep Conc	
rep.	unequal	unequal	10	unequal	
d.f.	49	49	49	49	
l.s.d.		0.04300X		0.06081	min.rep
	0.03331	0.03598	0.04300	0.05267	max-min
		0.02720		0.04300X	max.rep

(No comparisons in categories where s.e.d. marked with an X)

***** Stratum standard errors and coefficients [illegible]