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Effects of seed coating on germination and early seedling growth in cereals

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Preface

This thesis is based on studies conducted from September 2007 to September 2013 in the Department of Crop Production and Agroecology in the Tropics and Subtropics, Section: Crop Water Stress Management; University of Hohenheim, Stuttgart Germany and is submitted together with the enclosed three manuscripts in partial fulfillment of the requirements for Ph.D degree at the Faculty of Agricultural Sciences.

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Summary

Grain cereals such as barley, rye, wheat or sorghum, constitute a major share in human diet worldwide. Climate change threatens cereal production systems due to emerging unreliable rainfall patterns and thus, renders crop production vulnerable to early season failure of crop establishment. Breeding drought tolerant genotypes is a long and complicated process, thus not suited to respond to environmental changes quickly but rather to address the problem in a longer time frame. Seed coats increasing water availability to the seed and enhancing early vigor of the seedling may be a better short term solution. Seed coats containing hydro-absorbers such as Stockosorb® or Geohumus® can improve water availability and in combination with other substances for example humic acids or plant fortifiers such as Biplantol® may improve early vigor. However, very low germination rates have been observed in seeds coated in this way resulting in slow adoption of seed coating technology in agriculture.

The present study analyzed the effects of seed coating on germination, which seed coat composition can enhance germination rate and early vigor and why, the effects of seed coat on germination and seed physiology, and which influence seed coats containing different kinds of hydro-absorbers have on the drought resistance of seed during germination. Studies were conducted on barley, rye, and wheat seeds. In these species the mode of action of differently composed seed coats and the effects of differences in seed coat strength were studied whereas the studies on drought resistance were performed with sorghum seeds. The following results were obtained and submitted for international publication:

1) Seed coating in general reduced germination rates as compared to uncoated seeds. Seed coating thickness was the determining factor. With a share of the seed coat of 75% of the total grain (seed + seed coat) germination was very little affected by coating. However, smaller seed coat shares and particularly shares smaller than 50% of the total grain severely reduced germination rate. This effect was especially pronounced in wheat.

2) With seed coat shares larger than 75% and the coats dotted with different substances generally resulted in high germination rates. However, strong genotypic effects were observed in responses of seed physiology and dry matter partitioning to the different substances included in the coat, with barley generally responding positively, rye intermediate and in wheat generally the weakest effects were observed. Across genotypes Biplantol included in the coat mainly promoted shoot growth, humic acid increased root growth and hydro-absorbers mainly the rate of germination and early vigour. Due to those results only seed coated with hydro-absorber containing coat and with coat not smaller than 75% were used for all following studies.
3) Seedlings growing from coated seeds with coat shares > 75% showed accelerated early seedling growth with strongly reduced respiratory losses during the mobilization of endosperm reserves, combined with significantly increased mobilisation efficiency in all three cereals. Analyses showed that the sucrose metabolism and thus the availability of glucose as energy provider for growth differed strongly between coated and uncoated seeds as well as among the cereal species. Embryos from coated seed (particularly in barley and wheat) seemed to grow better with significantly less glucose indicating a chance in the enzymatic cleavage of sucrose that could only be due to the higher energy efficiency of the enzyme sucrose synthase.
4) Studies during germination *in sensu stricto* (the first 48h hours after soaking) showed that in the embryos of coated seeds conditions were hypoxic with oxygen concentrations of less than 5% of atmospheric oxygen as compared to 60-80% oxygen of atmosphere in embryos of uncoated seeds. From this it was deduced that the lower respiratory losses during germination of coated seeds are due to a switch in sugar metabolism from invertase based cleavage of sucrose to sucrose synthase based cleavage of sucrose which is the less energy demanding pathway in the near absence of oxygen.
5) A last study on drought resistance of coated seed whose coats comprised two different hydro-absorbers (Stockosorb or Geohumus) showed that the drought responses of coated seed differed little form uncoated seeds. However, seedling

growth under both drought and fully watered conditions was affected by the type of hydro-absorber in the coat. Whereas Stockosorb promoted rather root growth, Geohumus enhanced shoot growth.

In conclusion, it can be stated that seed coats investigated in this study in general promoted germination rate and success in cereals if the seed coat has the appropriate strength i.e. coat shares not below 75% of total grain. Additives such as Biplantol or humic acid promote vigour and influence dry matter partitioning in favour of specific organs which could be employed as management options during germination. It was shown that seed coats influence the germination and seedling metabolism und induce hypoxic conditions in embryonic tissue which shift the sugar metabolism to a more energy efficient pathway.

Oxygen dynamics in the different seed tissues require further studies and need to be better understood in order to employ the positive effects of seed coating in a targeted and species-specific approach to improve and enhance crop establishment particularly in drought prone cereal production systems. Another future pathway could be including nutrients in the coat that promote early seedling growth and for systems threatened by early drought spells or unreliable rainfall a seed coat that would conduct water to the seed only after soil moisture has surpassed a given threshold and thus induces germination only when water availability is optimal.

Zusammenfassung

Körnergetreide wie Gerste, Roggen, Weizen oder Sorghum, stellen einen großen Anteil der menschlichen Ernährung. Des klimawandel bedroht den Getreideanbau auf Grund sich verändernder Niederschlagsverteilungen, welche diese Systeme anfällig machen für Ertragsverluste auf Grund mangelhaften Feldaufgangs. Die Züchtung trockentoleranter Genotypen ist ein langwieriger und komplizierter Prozess und deshalb wenig geeignet, zügig auf Umweltveränderungen zu reagieren, sondern Züchtung leistet eher einen Beitrag zur langfristigen Lösung des Problems. Saatmäntel, die geeignet sind das Wasserangebot für den Samen zu erhöhen bzw. die Wüchsigkeit zu verbessern, können eine kurzfristige Lösung des Problems darstellen. Saatmäntel mit Hydroabsorbern beispielweise Stockosorb oder Geohumus können in Kombination mit anderen Substanzen wie Huminsäuren oder Pflanzenstärkungsmitteln wie Biplantol die Wasserverfügbarkeit und Wüchsigkeit verbessern. Allerdings haben geringe Keimungsraten von ummantelten Samen die Verbreitung und Akzeptanz dieser Technologie bisher verzögert.

Die vorliegende Arbeit beschäftigt sich mit den Fragen wie Mantelsaat auf die Keimung wirkt, welche Mantelzusammensetzung die Keimung befördert und warum, wie die Anwesenheit eines Saatmantels auf die Keimlingsphysiologie wirkt und welchen Einfluss Mäntel mit unterschiedlichen Hydroabsorbern auf die Trockenheitsresistenz von Saatgut während der Keimung haben. Die Untersuchungen wurden an Gerste, Roggen, Weizen und sorghumsaatgut durchgeführt. An diesen Arten wurde die Wirkungsweise unterschiedlich zusammengesetzter und unterschiedlich starker Mäntel untersucht wohingegen die Trockentoleranzuntersuchungen an ummanteltem Sorghumsaatgut durchgeführt wurden. Es wurden folgende Ergebnisse erzielt und entsprechend international zur Publikation eingereicht:

- Saatmäntel beeinträchtigten in allen Fällen die Keimungsrate im Vergleich zu Nacktsaat. Die Mantelstärke war hier ausschlaggebend. Wenn die Mantelstärke 75% des Gesamtkorngewichts (Saatkorn+Mantel) überstieg, wurde die Keimung nur noch

unwesentlich beeinflusst wohingegen Mantelstärken unter 50% die Keimungsraten stark reduzierten. Dieser Effekt war besonders bei Weizen ausgeprägt.

- Wenn Mäntel verwendet wurden, die 75% des Gesamtkorngewichtes überschritten und diese mit unterschiedliche Substanzen versetzt waren, dann war der Keimungserfolg immer gegeben aber die unterschiedlichen Substanzen wirkten bei den verschiedenen Arten unterschiedlich auf die Keimungsphysiologie. Generell lässt sich sagen, dass Biplantol vor allem das Sprosswachstum, Huminsäure vor allem das Wurzelwachstum und der Hydroabsorber vor allem die Keimungsgeschwindikeit und Wüchsigkeit positiv beeinflusst haben. Aufgrund dieser Ergebnisse wurden in den Folgeversuchen nur noch mit Mänteln gearbeitet die Hydroabsorber enthielten und mehr als 75 % Mantelanteil am Gesamtkorngewicht hatten
- Das derart ummantelte Saatgut aller drei Getreidearten zeigte ein beschleunigtes frühes Sämlingswachtum, mit stark reduzierten Respirationsverlusten während der Mobilisierung von Endospermreserven und damit verbundener signifikant gesteigerter Mobilisierungeffizienz. Die Analyse ergab, dass sich der Saccharosemetabolismus und die damit verbundene Bereitstellung von Glucose als Energieträger sowohl zwischen den Mantelvarianten als auch unter den Getreidearten unterschied. Embryos in ummantelter Saat (vor allen bei Gerste und Weizen) schienen mit deutlich weniger Glucose besser zu wachsen, was auf eine veränderte enzymatische Spaltung von Saccharose hindeutet, die nur in einer höheren Energieeffizienz des Enzyms Saccharosesynthase begündet sein konnte.
- Eine Untersuchung der frühen Keimung (die ersten 48h nach Imbibition) zeigte, dass im Embryo des ummantelten Saatgutes hypoxische Bedingungen vorherrschten mit Sauerstoffkonzentrationen von unter 5% der Aussenluft, im Vergleich zu etwa 60-80% der Aussenluft in Embryos der jeweiligen Nacktsaat. Daraus ließ sich ableiten, dass die geringere Respiration von ummanteltem Saatgut während der Keimung auf eine Umstellung im Zuckerstoffwechsel von Invertase-basierter Spaltung von Sacharose auf Sacharosesynthase-basierter Spaltung von Sacharose beruht, die mit deutlich geringeren Energieverlusten einhergeht.

- Eine abschliessende Untersuchung zur Trockenheitsresistenz von ummanteltem Saatgut, in dessen Mantel unterschiedliche Hydroabsorber eingearbeitet waren, zeigte dass sich das Trockenstressverhalten von ummanteltem Saatgut nicht bzw. nur unwesentlich von dem der Nacktsaat unterschied. Allerdings wirkten die Hydroabsorber unterschiedlich auf das Setzlingswachstum. Während Stockosorb im Wesentlichen das Wurzelwachstum förderte, verstärke Geohumus das Wachstum des Sprosses.

Zusammefassend lässt sich feststellen, dass die Mantelsaaten, die in dieser Arbeit untersucht wurden, im Allgemeinen die Keimung und den Keimungserfolg bei Getreiden verbessern. Es ist darauf zu achten, dass die Mäntel eine ausreichende Stärke haben. Zusatzstoffe wie Huminsäuren und Biplantol verbessern die Wüchsigkeit und verstärken die Biomasseverteilung zu Gunsten unterschiedlicher Organe und können so als Steuerungselemente mit genutzt werden. Die Mantelsaat greift in den Keimungs- und Sämlingsstoffwechsel ein und führt zu hypoxischen Bedingungen im embryonalen Gewebe, die den Zuckerstoffwechsel im Embryo zu Gunsten eines Energie effizienteren Stoffwechselweges verändern. Zukünftig müssen die Sauerstoffdynamiken in den unterschiedlichen Geweben des Saatkorns genauer untersucht und verstanden werden, um die positiven Effekte der Ummantellung zielgerichtet und artspezifisch zur Verbesserung und zur Steigerung der Verlässlichkeit des Feldaufganges bei Getreide einzusetzen. Zusätzlich sollte darüber nachgedacht werden Nährstoffe, die das frühe Sämlingswachstum befördern, in den Mantel zu integrieren und in trockenheitsgefärdeten Gebieten wäre ein Saatmantel von Vorteil, der als Funktion des Bodenwassergehalts Wasser erst bei Überschreiten eines Schwellenwertes an das Saatkorn leitet und der Keimling dadurch erst bei guter Wasserversorgung beginnt sich zu entwickeln.

1 General introduction

Big grain cereals like barley, maize, rye and wheat as well as sorghum, form a major bulk of human diet worldwide (FAO 1999; Patil 2007). Drought is generally accepted to be the most widespread abiotic stress experienced by crop plants and is a serious problem in many parts of the world where these cereals and other small-grained cereals form the staple diets (Quarrie *et al*. 1999). With changing environmental conditions due to climate change, regions where these cereals are grown are threatened by and vulnerable due to lack of means and the know-how to adapt swiftly to imminent changes in agricultural systems (Rosenzweig and Hillel 1998; Quarrie *et al*. 1999). Vast proportions of agricultural lands in these regions are coming under drought or experiencing intermittent drought spells in the beginning of the planting season and this problem is at least two pronged. First of all, there is a certain lack of reliable forecasts to approach system changes in a focused way and secondly, many systems, in particular, rain-fed systems with strongly contrasting seasons, suffer from increasingly unreliable rainfall patterns especially at the onset of the rainy season (Bates *et al*. 2008). Attempts to develop genetic traits that are drought tolerant are not only long termed, but also complicated because drought itself is a complex phenomenon with spatial specificity making for difficult analysis and interpretation of results obtained over time (Quarrie *et al*. 1999; Hlavinka *et al*. 2009).

Without in-depth knowledge for long term adaptation of cropping systems and cropping calendars, there is still a need to provide means to farmers to mitigate potential crop failure due to unreliable rainfall patterns early in the season as an intermediate measure to sustain production along more traditional systems in order to create a buffer to the time when more long-lasting solution are available. In addition to the unreliable and volatile rainfall patterns, most agricultural lands especially in the developing world are degraded (Bai *et al*. 2008), further complicating the situation. The use of hydro-absorbers as soil ameliorates which have the capacity to store moisture in the face of drought have been widely employed for the successful rehabilitation of degraded lands using trees (Dehgan *et* *al*. 1994; Sarvas *et al*. 2007; Hütterman *et al*. 2009). However, when crops were grown on soils containing these absorbers, no major significant improvement was observed because of the complex interaction between these polymers, soil and fertilizers (Ghebru *et al*. 2007; Nha 2012). As a consequence of these limitations, the use of hydrophilic materials or hydro-absorbers as coatings around seeds thereby increasing the amount of water available for germination and seedling development, is a promising approach to improving stand establishment under conditions in these regions (Berdahl and Barker 1980); given the fact that early establishment of crops is critical for achieving maximum yield and that drought is one of the most important constraints of biomass production (Willenborg *et al*. 2004; Soltani *et al*. 2006; Lukacs *et al*. 2008).

Coating technology was developed for cereal seeds in the 1930's by Germains (a British seed company). It has been used to achieve different purposes such as precision sowing, synchronise the flowering of male inbred parents with adjacent rows of female inbred seeds (Vyn and Murua 2001), to improve seed quality during storage, to protect young seedlings from low temperatures, diseases and pests (Schneider and Renault 1997; Sharratt and Gesch 2008). Also, different substances have been employed in coating seeds such as nutrients (Silcock and Smith 1982; Scott *et al*. 1987; Hassan *et al*. 1990; Mikkelsen *et al*. 1993; Scott 1998), hormones (Powell and Mathew, 1988), or peroxides to provide oxygen (Dahlani *et al*. 1992). Currently, polymer coating with hydrophilic materials (temperature activated coatings) from Landec Ag of Monticello, IN, USA has also been experimented extensively (Vyn and Murua 2001). This particular type of coat enables the seed to withstand persisting low temperatures at the beginning of the planting season in the northern United States Corn Belt, thereby ensuring stand establishment by limiting frost effects on germination (Vyn and Murua 2001; Sharratt and Gesch 2008; Willenborg *et al*. 2008). However, these temperature activated hydrophilic materials contain large amounts of acrylic polymers which are thought to be cancerous and harmful to soil microbial communities (Vyn and Murua 2001) although other findings report that biodegradation by fungi occurs in forest ecosystems (Hüttermann *et al*.

2009). Secondly, work on this temperature activated coatings is limited and focuses mainly on corn, soy bean and canola (*Brassica napus*) with little known on the effect on major cereals such as barley, rye and wheat.

As global warming continues, the scale of drought prone areas is increasing with water availability for crop growth limited, varied and unreliable leading to the necessity for the assessment of hydro-absorbers which have the ability to store water and make it available to growing seedlings during these periods of crisis. Some limited studies have been carried out in the past on mainly the germination rate and water uptake of coated seeds but setbacks encountered because coating drastically reduced germination rates (Berdahl and Barker 1980). Most of these researches focused on field trials and generalized conclusions of what coating effects on seedling growth are have been recorded (Vyn and Murua 2001; Sharratt and Gesch 2008; Willenborg *et al*. 2008). In-depth knowledge of what actually happens in seeds with hydro-absorber coating with regards to starch mobilization, sugar metabolism and the amount of oxygen available during the different phases of germination and early seedling establishment, as well as technical aspects such as the effects of different coating shares (amounts) and composition on germination rate, seedling establishment, mobilisation efficiency and the responses of different cereals to coating technology, remains at large. This study which focused on barley, rye, sorghum and wheat, was carried out within the framework of the project ‘Batros’ (Bodenmelioration und Anbauverfahren für trockenheitsgefährdete Standorte) under the umbrella of ‘Klimazwei’ with the following hypothesis

1.1 Hypotheses

I. Coatings significantly affected seed germination, partitioning and seedling establishment.

II. Imbibition and oxygen transfer to embryo is better in the presence of additional coatings.

III. The amount of oxygen is reduced in the presence of coatings.

IV. Hydro-absorber coatings affect enzyme and invertase activity in germinating seeds by influencing reserve mobilization.

V. Hydro-absorber coatings minimize the effects of a drought during early seedling development in sorghum.

1.2 Objectives

The objectives of this work were:

a) To assess the effects of different coatings and coat shares on seedling growth and dry matter partitioning during germination

b) To analyze the effects of coating on imbibition rate and capacity and this affects available water to the growing seed

c) To measure the amount of oxygen in the embryo as germination progressed

d) To investigate invertase activity under coated compared to uncoated conditions

e) To analyze the effects of coatings during early drought stress in sorghum

We obtained barley, rye, sorghum, and wheat seed materials from the company, Freudenberger® (http://www.freudenberger.net). Seed batches comprised of uncoated and coated seeds. Seeds were coated with the following:

1. Biplantol®: It is said to be a plant fortifier believed to promote soil life, root development (Terhoeven-Urselmans 2002/04) and increase plant weight (Zimmermann and Mathis 2002).

2. Humic acids. It is believed to promote root development (Cooper *et al*. 1998; Arancon *et al*. 2006), plant growth especially under stress conditions (Asik *et al*. 2009) and affect nutrient uptake (Cooper *et al*. 1998; Nardi *et al*. 2002).

3. Hydro-absorbers which was either be Stockosorb® or Geohumus®

    a) Stockosorb: It is a cross-linked potassium based polyacrylate/ polyacrylamide hydrophilic polymer which is nutrient free (Dehgan *et al*. 1994; Ghebru *et al*. 2007).

    b) Geohumus: Twenty five percent is made up of organic cross-linked, partially neutralized polyacrylic substances and 75%; mineral components: ground rock, minerals and washed sand (Nha 2012). It is marketed as able to absorb and store water forty times its weight.

4. A combination of Biplantol®, humic acids and Stockosorb® referred to as ‘’ALL’’

Presentation of seeds

a) Uncoated seeds

Barley

Rye

Wheat

Susu and Piper (sorghum seed)

Susu

Piper

b) Coated seeds

ALL	Absorber	Biplantol	Humic Acid

Barley

Rye

Wheat

Sorghum

1.3 Background and pre-trials

At the beginning of our work, we sowed barley, rye and wheat seeds which were either coated or uncoated in an effort to check for germination rates but results between replicates were varied and inconsistent. Closer observation when coated grains were germinated in a sequence with increasing amount of coating revealed that the coating amounts might play a role in the germination rate. This prompted us to do a 1000-grain weight of the uncoated seed in order to obtain the mean grain weight for all the cereals (chapter 1). From this mean grain weight, the coated seeds for each cereal and all treatments were sorted into 3 classes based on the amount of coat surrounding the seed within, thus:

a) Coat share less than 50 %

b) Coat share 50-75 %

c) Coat share greater than 75 %

See paper 1 for further details. Coated together with the uncoated grain were germinated on moist filter paper and considered germinated when a tiny radicle was seen to have emerged. Low germination rate is known as a major setback in coated seeds. Therefore, it was very important to determine a germination cut-off period. In order to determine the length of the germination period, seeds that did not germinate over time were subjected to tetrazolium test (1 % w/v; 2, 3, 5- triphenyl-tetrazolium chloride; Merck® Darmstadt, Germany) in order to check whether the embryo was still viable or not, since this could not be determined with the naked eye. The cut off for barley, rye and wheat were determined to be ten days after soaking (fig 1).

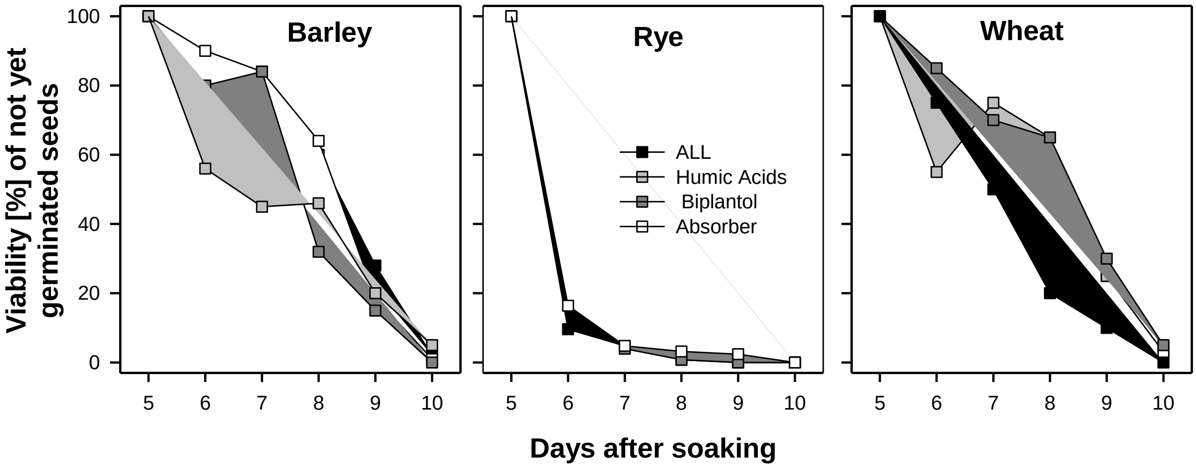

Fig. 1 Percentage of viable barley, rye and wheat seeds over time. All the seeds were tested with the 1 % w/v; 2, 3, 5- triphenyl-tetrazolium chloride following standard procedures.

This was a cumulative dissertation composed of four papers which are presented in chapters. We began by addressing the effects of coat composition and shares on mobilization efficiency (chapter 1). The results obtained then led us to some questions and issues which are addressed in the subsequent chapters.

2 Effects of composition and share of seed coatings on the mobilization efficiency of cereal seeds during germination

[The content of this chapter has been published in the Journal of Agronomy and Crop Science `` Gorim, L. and Asch, F. 2012: Effects of composition and share of seed coatings on the mobilization efficiency of cereal seeds during germination. Journal of Agronomy and Crop Science 198 (2): 81-91'']

Abstract

Cereal production systems are increasingly threatened by suboptimal water supply or intermittent drought spells early in the planting season. Seed coated with hydrophilic materials or hydro-absorbers that increase the amount of water available for germination and seedling development, is a promising approach to improving stand establishment under changing conditions. Barley, rye and wheat grains with combinations of hydro-absorber, humic acid, and Biplantol® in different shares of the total seed mass were germinated in plates at 25 °C on moist filter paper. Germination rates, resource partitioning, and mobilisation efficiency were assessed and compared to uncoated seeds. Results show a strong influence of coat thickness and composition on the germination rate and the efficiency of mobilisation of carbohydrates stored in the endosperm. In general, coating significantly reduced germination rate and total germination as compared to uncoated seeds in all cereals tested. Differences in coating thickness had a distinct effect on germination rate for most combinations of coatings and species. Germination rates increased with increasing coat size. This effect was most pronounced for coatings containing hydro-absorbers and least pronounced for coatings containing humic acid or Biplantol®. Coating generally increased the amount of carbohydrates partitioned to the roots and thick coating increased the efficiency of grain reserve mobilization compared to the

uncoated seeds. Differences between species and the implications for coating related changes in germination metabolism are discussed.

2.1 Introduction

Cereals form the major food source for the majority of the world’s population (FAO 1999). The increasingly imminent climate change and the increasing demand for food by a growing world population profoundly influence production systems in several ways (FAO 2003; Fischer *et al*. 2002). On the one hand, areas so far not used for agricultural production maybe brought into cereal production because either the environmental conditions become more favorable or pressure on land resources forces more marginal lands into production (Tilmann *et al*. 2001). On the other hand, well established cereal production systems may be shifting out of the most productive window, by climate change induced changes in annual water availability, changes in the seasonal cardinal temperatures, or both (Olesen *et al*. 2011; Rosenzweig and Hillel 1998). All those situations require cereals with a relatively high degree of tolerance to abiotic stresses such as drought, salinity or detrimental temperature ranges. Cereals such as barley, rye, and wheat possess a high degree of abiotic stress tolerance and form a major bulk of human diet worldwide (Ortiz *et al*. 2008; FAO 1999).

Cereal production systems, most importantly for maize and rice, are increasingly threatened by persistent suboptimal water supply (Rosenzweig *et al*. 2007) or intermittent drought spells early in the planting season leading either to unsuitability of the chosen crop or to an increase in the risk of crop failure (Hlavinka *et al*. 2009). This problem is at least two pronged: 1) there is a certain lack of reliable forecasts to approach system changes in a focused way, and 2) many systems, in particular rainfed systems with strongly contrasting seasons, suffer from increasingly unreliable rainfall patterns especially at the onset of the rainy season (Bates *et al*. 2008). Without in-depth knowledge for long term adaptation of cropping systems and cropping calendars being available yet, there is a need to provide farmers with means to mitigate potential crop failure due to unreliable rainfall patterns early in the season as an intermediate measure to sustain production along more traditional systems. Using alternative crops and improved seeding and seedling management may create a transitional economic buffer to the time when a more long-lasting solution will be available.

Seed coated with hydrophilic materials or hydro-absorbers that increase the amount of water available for germination and seedling development, is a promising approach to improving stand establishment under changing conditions (Berdahl and Barker 1980), given the fact that early establishment of crops is critical for achieving maximum yield and that drought is one of the most important constraints of biomass production (Willenborg *et al*. 2004; Soltani *et al*. 2006; Lukacs *et al*. 2008).

The technology for coating cereal seed was developed in the 1930's by Germains (a British seed company). Subsequently, it was employed for different purposes such as precision sowing, synchronise the flowering of male inbred parents with adjacent rows of female inbred seeds (Vyn and Marua 2001), to improve seed conservation during storage, or to protect young seedlings from low temperatures, diseases, and pests (Sharratt and Gesch 2008). Seed coats may contain specific substances such as nutrients (Silcock and Smith 1982; Scott *et al*. 1987; Hassan *et al*. 1990), peroxides to provide oxygen, or hormones (Powell and Mathew 1988). In drought prone areas, coatings enhanced with water absorbing polymers may help to mitigate spatial and temporal water deficiencies in the critical germination and seedling growth phases. However, the effects of water absorbing polymers or growth promoting substances on germination and seedling establishment needs to be thoroughly assessed before such a technology can be proposed for any cropping system as a stability increasing approach. To date, there is no information available about the effects these coatings may have on the germination and early seedling growth of major cereals. Therefore, in this study we assessed the effects of including a hydro-absorber (Stockosorb®), having the ability to store water and make it available to growing seedlings (Berdahl and Barker 1980), as part of a seed coat on germination rate, seedling growth, and mobilisation efficiency in three different cereals. Other than just increasing water availability surrounding the seed, seedlings can profit from fast absorption of water into the seed through increased germination speed and promotion of early root growth, thus improving the seedlings access to water and nutrients. Therefore, seed coats used in this paper were augmented in addition with Biplantol®, an organic, homeopathic product supposed to promote root development (Bioplant Naturverfahren GmbH, Konstanz, Germany) and humic acid, known to improve water penetration into seeds, promote germination, and stimulate root growth (Tan and Nopamornbodi 1979; Tattini *et al*. 1991; Gonet *et al*. 1995; Mackowiak *et al*. 2001; Atiyeh *et al*. 2002).

There are no reports in the available literature on the effects of the substances mentioned above on the germination and early seedling physiology of barley, rye, and wheat. Equally nothing is known on the minimum thickness of the seed coat required to have any effect. Therefore, the aims of this study were to assess in all three cereal species (1) the effects of different seed coats on germination rate of different cereal seeds, (2) the effects of coating thickness on germination and early seedling development, and (3) the effects of coating on the remobilisation efficiency for grain reserves and early root development.

2.2 Materials and Methods

2.2.1 Plant materials and treatments

Seeds of spring barley (*Hordeum vulgare* L., cv. Maltasia), winter rye (*Secale cereal* L., cv. Jobaro), and spring wheat (*Triticum aestivum* L., cv. Thasas) were obtained from Freudenberger Feldsaaten GmbH and used in all experiments. Seeds were natural (uncoated) or coated with the coating Mantelsaat® developed by Freudenberger Feldsaaten GmbH dotted with different chemical substances, resulting in 5 treatments: (1) Uncoated seed, (2) coat dotted with hydro-absorber (Stockosorb®), (3) coat dotted with Biplantol® PROVIDER, (4) coat dotted with humic acid PROVIDER and (5) a combination of 2, 3 and 4. The coated seeds are referred to as the ‘grain’ whereas the uncoated seeds and the seeds inside the grains are referred to as ‘seed’. The viability of the seeds was tested over the germination period using the tetrazolium test (1% w/v; 2, 3, 5- triphenyl-tetrazolium chloride; Merck® Darmstadt, Germany) according to the method of ISTA (Anonymous,2010) in order to determine the length of germination period for all treatments and cereals (data not shown).

2.2.2 Determination of germination rates and early seedling dry matter

Random samples of 50 seeds or grains were taken for the 5 treatments and for each cereal. They were individually weighed and placed on filter paper moistened with 12ml distilled water (Ecolab-Bogen-Filterpapier, Neolab® Heidelberg, Germany) in PET plates of 19.5 x 19.5 cm, their position noted, and germinated in a growth chamber (Percival Scientific, Inc. Iowa, U.S.A) at 25 °C with a 12 hours photoperiod. Grains and seeds were spaced 3 x 2.5 cm in the plates with each treatment replicated three times within each experiment which was each repeated three times. To ensure that the filter paper remained moist, 10 ml distilled water was added into each plate daily. A grain or seed was considered germinated when a radicle longer than 1 mm was visible. The number of newly germinated grains or seeds was recorded daily for the different treatments and the cumulative germination rate was calculated. For the determination of seedling dry matter, the same experiment was set up once again but with more replicates to ensure that a sufficient number of seedlings of the same physiological age could be sampled throughout the duration of the experiment. The mass and the position of the individual grains or seeds in the plates were noted as well as the date that they germinated. Seedlings were sampled for dry matter determination during the germination test and for each seedling; the time span between germination and sampling was recorded. This individual time recording was necessary to standardize physiological age of seedlings since germination was irregular especially in the case of the absorber coated seeds.

Dry matter determination started on the third day after soaking and continued daily until the eighth day after germination commenced. Seedlings were oven-dried at 70 °C for 24 h prior to weighing.

2.2.3 Grain and seed mass distribution

The grain and seed mass distribution were determined by weighing the individual grains or seeds of a 100 g sample per species and treatment using a fine balance (Precisa Gravimetrics AG Dietikon, Switzerland). From the distributions for the seeds, the average seed mass was estimated for each cereal. The assumption was made that all seeds inside the grain have exactly the average seed mass. Then for each grain, the coat share could be calculated as a percentage according to:

$$Coat\ share\ (\%) = \frac{\text{Mass of the individual grain} - \text{average seed mass}}{\text{mass of the individual grain}} \text{x } 100$$

The frequency distribution of the coat shares were plotted for each species and coating treatment. Grains were grouped according to their coat shares into 3 classes; (1) coat share <50 % (2) coat share 50-75 %, and (3) coat share >75 %.

2.2.4 Determination of reserves mobilization of grains

Grains from all three coat share classes were germinated and allowed to grow over a period of eight days as previously described above. Three seedlings of the same physiological age were sampled each day and divided into root, shoot, and grain. The coating was washed off from the grain and remaining seed as well as the other seedling parts were oven dried at 70 °C for 24 h for the determination of dry masses. The mobilization of grain reserves was calculated by using the average seed mass from the 100 g samples as:

$$Reserves\ mobilization\ (\%) = \frac{[\text{daily root dry mass} + shoot\ dry\ mass]\ \text{of the seedling}}{\text{average seed mass of the species}}$$

In addition, the shares of the dry matter gains was calculated for root and shoot as the daily dry matter gain of the respective organ related to the daily dry matter gain for both organs and expressed as a percentage. Photosynthesis and respiratory losses were ignored here but will be addressed in subsequent reports.

2.2.5 Statistical analysis

Least significant differences were calculated with an analysis of variance using the LSmeans statement in the general linear model (GLM) procedure of SAS.

2.3 Results

2.3.1 Effects of coating on germination rate and early seedling growth

In general, coating significantly ($p<0.05$) reduced germination rate and final germination as compared to uncoated seeds in all cereals tested (Fig. 1 a-c). In barley, the coatings did not significantly differ in affecting germination rates (Fig.1a), but they significantly reduced ($p<0.05$) root and shoot dry masses in most cases compared to the uncoated seed (Fig. 1d). In rye, coatings containing Biplantol® resulted in significantly higher ($p<0.05$) germination rates when compared with the other three treatments (Fig. 1b). Early root and shoot dry masses were significantly promoted by coatings containing Biplantol® or humic acid, but were not significantly reduced by coatings containing hydro-absorber when compared to the uncoated seeds. Combining all substances in one coating (Fig. 1e – ALL) did not off-set the growth reducing effect of the hydro-absorber. In wheat, coating effects on germination rate were strongest (Fig. 1c).

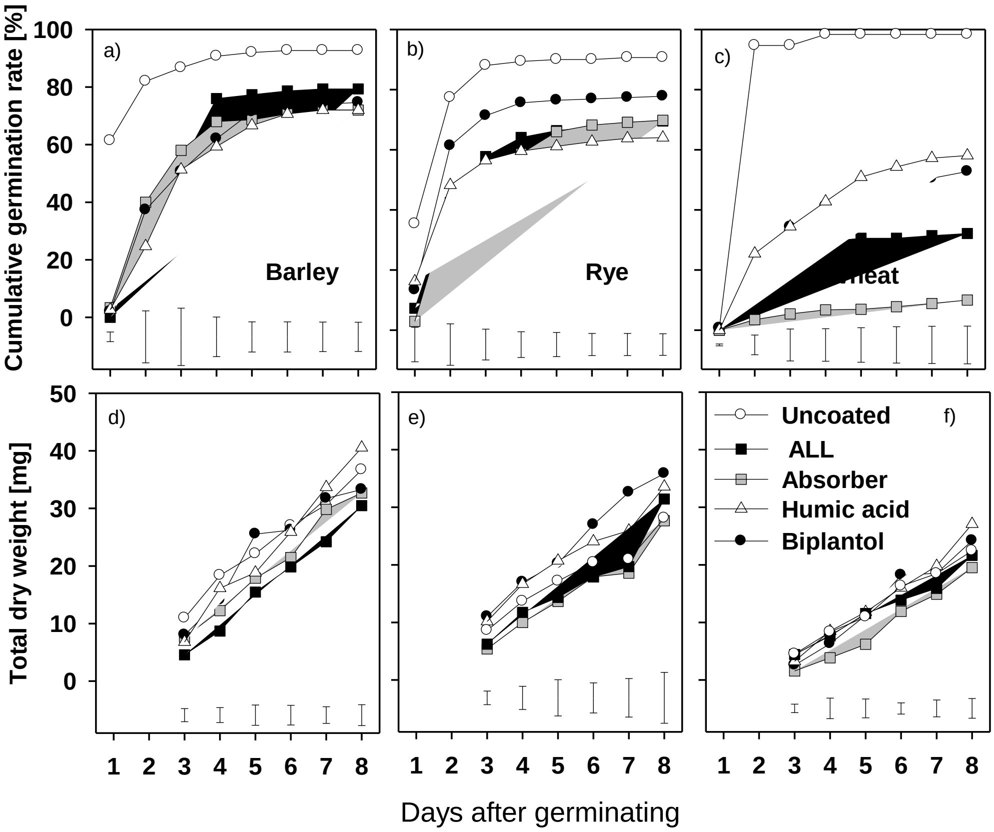

Fig. 1 Effects coating composition on germination rate and root and shoot (= total) dry mass of coated grains of barley, rye and wheat as compared to uncoated seeds. Bars indicate the least significant difference at α = 0.05.

Coatings containing hydro-absorbers severely reduced germination rate to about 10 % with hydro-absorber as single substance added to the coating and to about 25 % when all substances were combined in the coating. With Biplantol® or humic acid added to the coating, germination rate was less affected but still significantly ($p<0.05$) more reduced in wheat than in barley or rye. The strong coating effects on germination rate were not reflected in early root and shoot dry masses. Although early root and shoot dry masses in wheat were in general lower than in barley and rye, coatings did not significantly affect them as compared to uncoated seeds (Fig. 1f).

2.3.2 Grain mass distribution and coat share

Detailed observations of germination kinetics implied an effect of grain size on germination rate and root and shoot dry masses (data not shown). Grain size is dependent on the individual seed mass and the thickness of the coating.

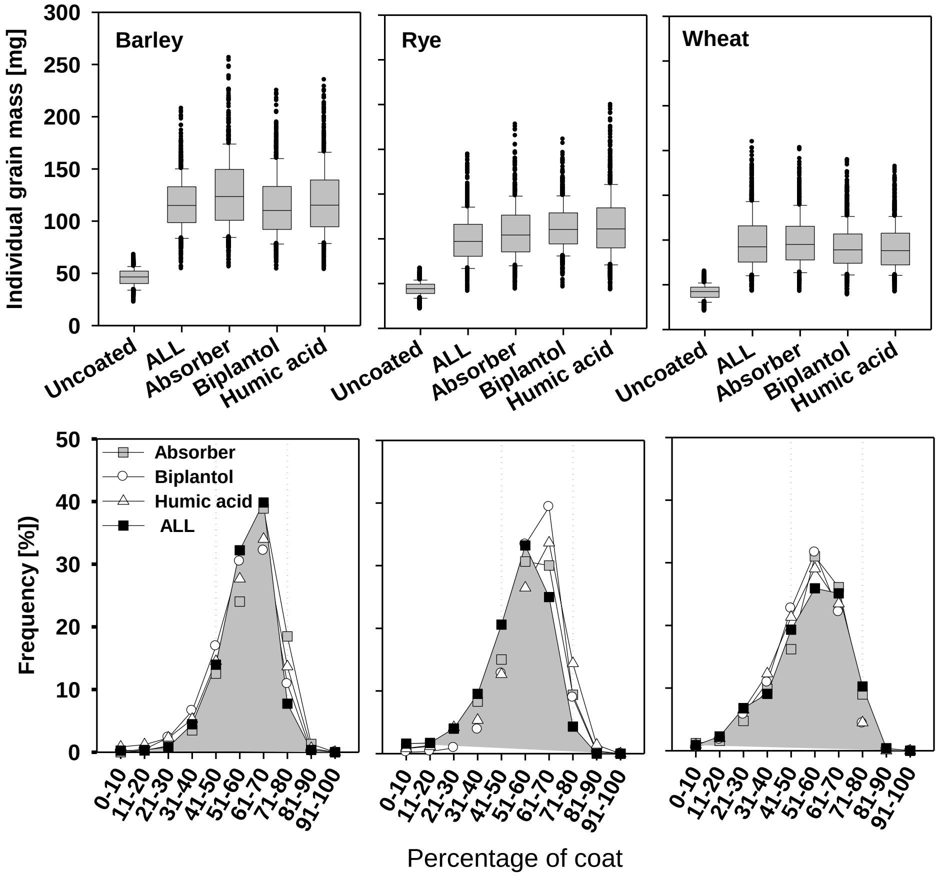

Fig. 2 Box plots of mass distributions for coated grains and uncoated seeds where a, b, c = mean with accompanying bar being the standard error for coat shares >75 %, 50–75 % and <50 % assessed during our study (upper part) and the frequency distribution of the coat share of coated barley, rye and wheat grains (lower part). Vertical dotted lines indicate the borders between the three coat share classes <50 %, 50–75 % and > 75%.

For each of the 5 treatments, grain mass distribution of a 100 g sample was determined (Fig. 2 top). Uncoated seeds of the three cereals had similar mass distribution patterns with a low variability. For each cereal, the average seed mass was determined and used for calculating the coat share percentage for the coated grain. Grain mass was generally 2 – 7 times higher than seed mass for all treatments and species and the variability in grain mass was high (Fig. 2 top). The average grain mass used in this kinetic study compared to that from the 100 g mass count distribution is shown in Figure 2 (top). The frequency distributions for grain mass are shown in Figure 2 (bottom). The frequency distributions were similar for the different coatings in each species and allowed to form three classes; <50 %, 50-75 %, and >75 % coat share.

2.3.3 Effects of coat share on germination rate

Coat shares had a distinct effect on germination rate for most combinations of coatings and species (Fig. 3). In barley, germination rates significantly ($p<0.05$) increased with increasing coat shares. This effect was most pronounced for coatings containing hydro-absorbers and least pronounced for coatings containing humic acid or Biplantol® (Fig. 3 a-d). Coat shares larger than 75 % of the total grain size containing absorbers resulted in germination rates close to those of uncoated seed (Fig. 1a), whereas coat shares <50 % severely reduced germination rates (Fig. 3 a,b). For rye, a similar pattern was observed (Fig. 3 e-h). But in contrast to barley, medium and large coat shares did not affect germination differently for the different coating compositions. In wheat, however, the effect of coat share on germination rate depended strongly on coating composition (Fig. 3 i-l). Coatings containing absorber alone (Fig. 3 j) severely reduced germination rate independent of coat share. Coatings containing Biplantol® or humic acid with coat shares <50 % and 75 % severely reduced germination rate (Fig. 3 k,l), whereas germination rates for medium coat shares were significantly higher when compared with the non-stratified sample (Fig. 1). With all substances combined in one coating (ALL – Fig. 3 i), coat shares >75 % resulted in final germination rates of about 80 % in wheat, whereas medium and small coat shares resulted in final germination rates of 50 and 18 %, respectively.

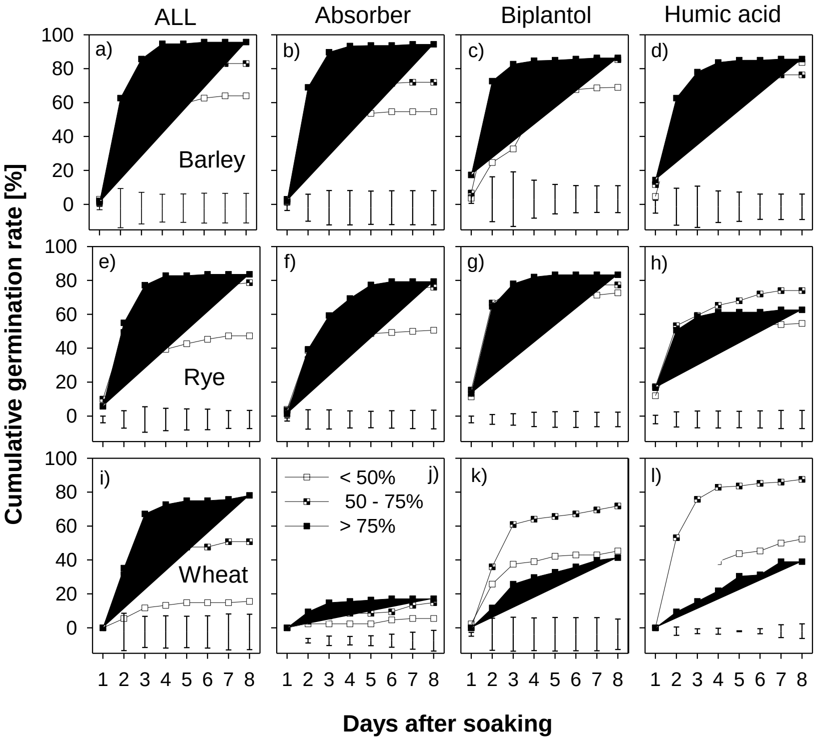

Fig. 3 Germination rates of coated barley, rye and wheat grains as dependent on the coat composition and the three coat share classes. Bars indicate the least significant difference at a = 0.05.

2.3.4 Effect of coating composition and coat share on early seedling growth

Coat share specific germination implied differences in early vigor and growth of different organs (data not shown). Differences in vigor during early seedling growth can be the result of either a better, more efficient mobilization of grain reserves, or an earlier onset of photo-autotrophy. Full autotrophy is achieved when the growing plantlet reaches its original seed weight, having thus compensated for respiratory weight losses during germination (Asch *et al*. 1999). To assess the effect of coat size and composition on early seedling growth, daily remobilization of grain reserves was calculated as described above and then plotted cumulatively for all coatings and species in Fig. 4 – 6.

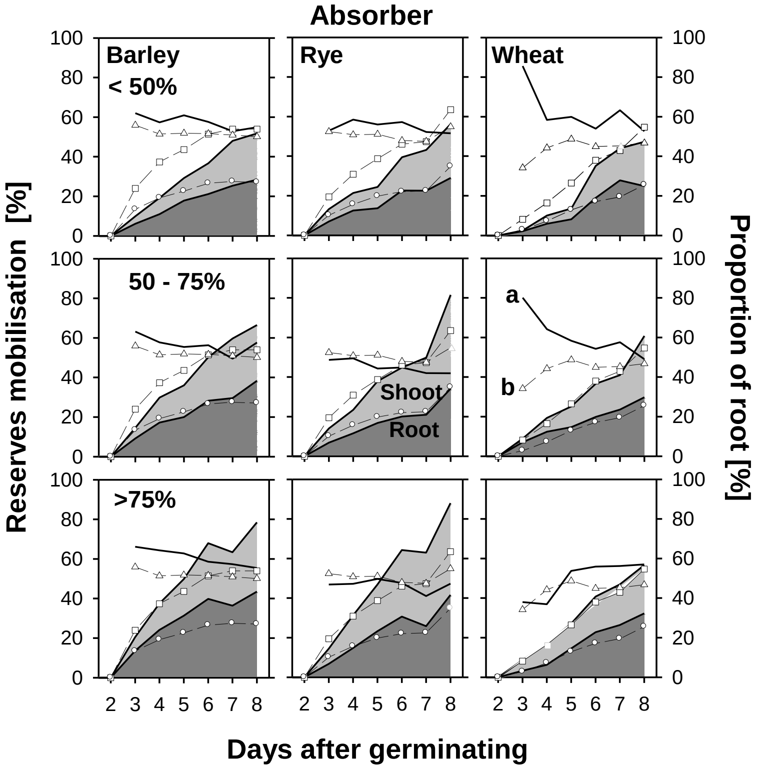

Fig. 4 Dry matter mobilization and proportions of root and shoot dry matter gains for grains coated with the hydro-absorber, Stockosorb®, compared to the uncoated seed. (a) proportion of root (%) for the treatment, (b) proportion of root (%) for the uncoated seed. Solid lines and shading indicate the proportion of root and shoot of dry matter mobilized from the grains. Broken lines and open symbols indicate the proportions of root and shoot dry matter mobilized from the uncoated seed.

In general, both coating composition and coat share strongly influenced early seedling growth and dry matter partitioning among organs when compared with uncoated grains. The cereal species tested responded differently to different combinations of coating composition and coat share. With few exceptions, coating increased the root/shoot ratio in the young seedlings as compared to seedlings from uncoated seed (Fig. 4-6). In wheat, coating composition and coat share had no effect on reserve remobilization as compared to seedlings from uncoated seeds. In wheat, in all cases about 60 % of the original grain mass was remobilized after 8 days, with dry matter preferentially partitioned to the roots in seedlings from coated grains (Fig. 4-6).

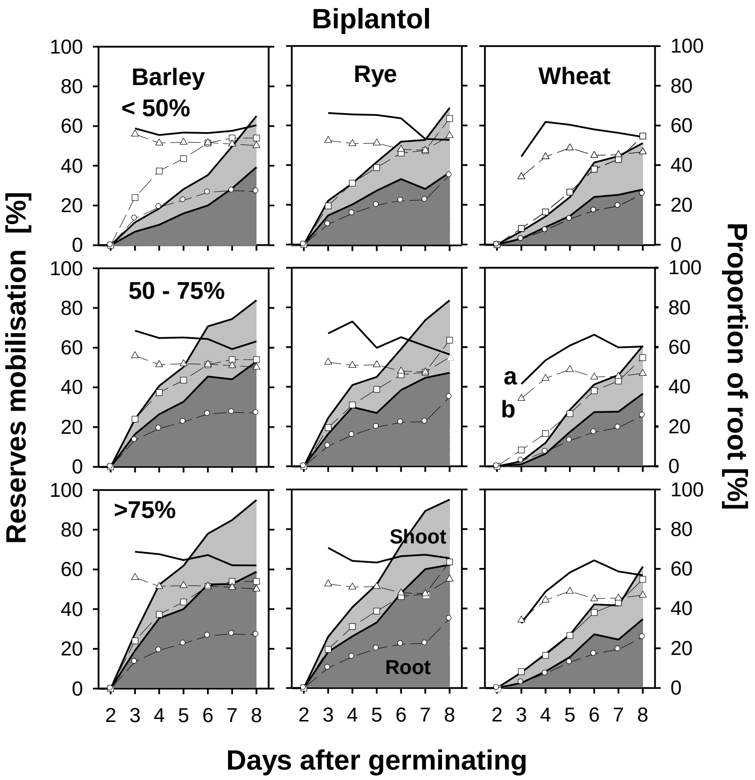

Fig. 5 Dry matter mobilization and proportions of root and shoot dry matter gains for grains coated with the plant fortifier, Biplantol®, compared to the uncoated seed. (a) proportion of root (%) for the treatment, (b) proportion of root (%) for the uncoated seed. Solid lines and shading indicate the proportion of root and shoot dry matter mobilized from the grain. Broken lines and open symbols indicate the proportions of root and shoot of dry matter mobilized from the uncoated seed.

With the exception of wheat, coatings containing hydro-absorber slowed down reserve remobilization when coat share was <75 % of the total gain. Coat shares >75 % promoted reserve remobilization as compared to seedlings from uncoated seeds from 4 DAS onwards, resulting in about 20 % increase in remobilization after 8 days of growth in barley and rye (Fig. 4).

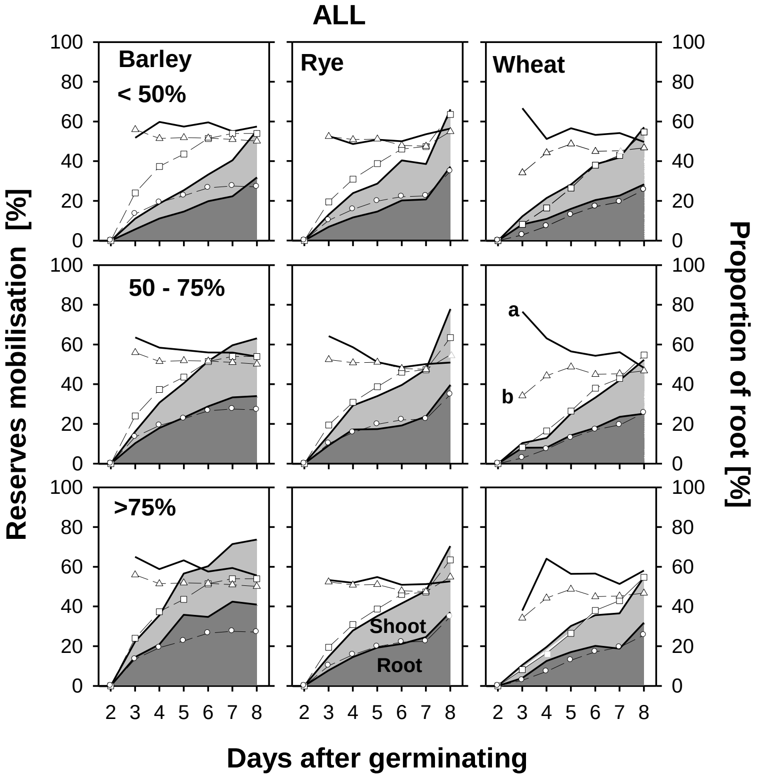

Fig. 6 Dry matter mobilization and proportions of root and shoot dry matter gains for grains coated with Stockosorb®, Biplantol® and humic acid compared to the uncoated seed. (a) proportion of root (%) for the treatment, (b) proportion of root (%) for the uncoated seed. Solid lines and shading indicate the proportion of root and shoot of dry matter mobilized from the grains. Broken lines and open symbols indicate the proportions of root and shoot dry matter mobilized from the uncoated seed.

The effect of coatings containing Biplantol® on remobilization differed between barley and rye only for coat shares <50 % where remobilization was slowed down in barley and not affected in rye as compared to seedlings from uncoated seeds (Fig.

5). Coating containing Biplantol® promoted preferential partitioning of biomass towards the roots in barley and rye for all coat shares, accelerated remobilization, and increased remobilization to 85 % and 95 % after 8 days for medium and large coat shares respectively. Coating containing humic acid induced results very similar to those with coatings containing Biplantol® in all three species. The only significant difference was observed in biomass partitioning towards the root in rye with coat sizes <50 % and >75 %, which did not promote preferential root growth as compared to seedlings from uncoated seeds (data not shown).

When all substances were combined in a single coating, effects of coat share on remobilization and dry matter partitioning were again different from those induced by single substance coating in all cereals but wheat (Fig. 6). Coatings containing all substances (Fig. 6) promoted preferential partitioning of biomass to the roots to a significantly ($p<0.05$) lesser extent than the single substance coating with humic acid or Biplantol® (Fig. 5). Remobilization was accelerated and improved in barley only with coat shares >75 %; was not or little affected for large and medium coat shares in rye and wheat, and was slowed down for coat shares <50 % in barley and rye (Fig. 6)

2.4 Discussion

2.4.1 Coating composition and coat share effects on germination

Coating seeds with potential germination promoting substances has been practised for several decades now. A review of the results available did not yield a clear picture of the effectiveness of coating in promoting germination. Seed coats seem to interact with a variety of factors such as soil type, nutrient availability, cultivation methods as well as species and these interactions depend on the type of coating and its composition (e.g. Richardson and Hignight 2010; Gorim *et al*. 2009; Peltonen-Sainio *et al*. 2006). In this study we investigated the germination of barley, rye and wheat seeds coated with Mantelsaat® (Feldsaaten Freudenberger 2011) including either Stockosorb®, humic acids, Biplantol® or a combination of all three substances as compared to uncoated seeds. For the uncoated seeds, final germination rates of up to 90% generally occurred within 4 days while this period was extended for coated grains in most cases as was shown before by Vyn and Marua (1980), Willenborg *et al*. (2004) and Sharratt and Gesch (2008), for temperature activated polymer coatings in soybean, canola, and corn.

Stockosorb® is a potassium based nutrient free hydrogel absorbing and retaining water of up to 400 times its weight. This hydrogel was first developed as a soil ameliorant to mitigate seasonal drought effects (Sarvas *et al*. 2007; Dehgan *et al*. 1994) but was shown to be impractical and costly as sometimes more than 80 times its recommended rate had to be applied to be effective (Ghebru *et al*. 2002). Stockosorb® contains a smaller amount of acrylic polymers and is, therefore, regarded eco-friendly (Ghebru *et al*. 2007) and when applied as a seed coating, potentially buffers water deficits during germination and early seedling growth. In the experiment presented here coating in general reduced the germination rate in all species with the level of reduction depending on the composition of the coating and on the species (Fig. 1). Whereas in barley the reduction in germination rate was lowest for all coatings, rye and wheat responded to coatings containing Stockosorb® with germination rates reduced by 20 % and 90 % respectively. These results are partly in line with Berdahl and Barker (1980) who reported seed coating with absorbers resulting in low germination rates in Russian wildrye. Coatings comprising either humic acid or Biplantol® did not significantly influence germination as compared to coatings containing absorber, except for wheat seeds where reductions in germination were less severe when coats did not contain Stockosorb®.

The germination rate depended strongly on the amount of coating material applied to the seed. In general, coatings shares of more than 75 % total grain mass had little effect on germination rates as compared to the uncoated control seeds whereas smaller amounts of coating material generally resulted in severe reductions in germination rate (Fig. 3). In contrast, wheat generally responded to large coat shares with a severe reduction in germination rate, independent of the coats' composition.

Only the combination of all substances in one coat showed some improvement in germination rate in wheat, which may have been due to the incorporation of humic acids in the coat as humic acids have been reported to promote germination (Loffredo *et al*. 2005; Türkmen *et al*. 2004). In most of the cereals tested, seed coatings containing humic acid with coat shares of 50 – 75 % showed better germination rates than those with large coat shares (>75 %). This confirms earlier reports showing that higher concentrations of humic acids resulted in lower germination rates with best rates obtained at humic acid levels of e.g. 640 ppm in corn (Tan and Norpamornbodi 1979).

2.4.2 Coating composition and coat share effects on early biomass partitioning

All coatings increased dry mass accumulation in young seedlings as compared to the uncoated seed when the coat share was greater than 75 % in barley and rye. In these cases, about 60 % of the total mobilised resources went into the roots and the proportion of roots was greater than 60 % in most cases which confirms results from earlier works (Gonet *et al*. 1995; Ayuso *et al*. 1996; Atiyeh *et al*. 2002). This effect was also observed for medium coat sizes containing Biplantol® or humic acid but not for any combination of coating substances including Stockosorb® or coat shares smaller than 50 %. Early root development is a desirable trait for seedling establishment, particularly under variable water supply. Despite the fact that germination rate was negatively affected by coating; for those seeds that germinated, coating generally increased the share of dry matter gains in the roots. This was particularly significant when seed coating included Biplantol® which has been described as an agent promoting root development by its manufacturers (Bioplant Naturverfahren GmbH).

2.4.3 Coating effects on grain reserve mobilization

For germinating grains to establish into fully functional seedlings, vigorous growth through fast and efficient mobilization of grain reserves as well as an early onset of productive photosynthesis is required (Asch *et al*. 1999). The efficiency of grain reserve mobilization differed strongly among the species, with the composition of the coating, and with the coat share. For example, remobilization efficiency 5 days after germination commenced for seeds coated with all test substances comprised in the coating (ALL – Fig. 6) was for coat shares smaller than 50 %, about 25 % of total grain reserves which corresponded to a 40 % reduction of mobilization efficiency compared to the uncoated grain in barley, 38% reduction in rye, and no change in efficiency in wheat. Coat shares larger than 75 % of the total grain, however, increased the mobilization efficiency in barley to about 38 % above the uncoated seed and in the other two cereals to the same level as in the uncoated seed. Increases in mobilization efficiency as compared to the uncoated seed, were even more pronounced for large coat shares in barley and rye when coatings contained Stockosorb® or Biplantol® alone. Grains coated with large amounts of coating material showing more efficient mobilization may be due to their ability to attract more moisture than grains with thinner coatings (Sarvas *et al*. 2007). As shown earlier for spring barley (Gorim and Asch 2010), coating thickness also influences the timing of cardinal points during germination such as onset of photosynthesis and fully autotrophic growth. Coat shares larger than 75 % of the total grain shortened the time to reach photo-autotrophy in barley by almost 5 days while minimizing respiratory losses during germination and increasing mobilization efficiency by more than 40 % as compared to the uncoated seed. Saturation with moisture in the coating material may lead to oxygen starvation of the developing embryo which may lead to a switch in the activity of the enzymes involved in starch breakdown and sugar transport. Switching from energy intensive invertase driven metabolism to energy conserving sucrose synthase driven sugar metabolism may reduce respiratory losses and increase mobilization efficiency (Koch 2004). Perata *et al*. (1997) reported an increase in sucrose synthase during germination under anoxic conditions in wheat and barley. This raises the question why in this study wheat seed coated with any substance or thickness responded with reduced germination rates and reduced mobilization efficiency and barley did not. Studies are under way investigating the effect of the different coatings and coat thicknesses on the imbibition rate of coated seed and the oxygen delivery to the embryo.

2.5 Conclusions

This study has shown that seed coating has a strong negative influence on germination rate. Effects on early dry mass partitioning were varied with species, coat composition and coat share. The coat share has a significant influence on germination rate and the capacity of the seedling to mobilize grain reserves. Carefully composing the seed coat may influence the partitioning between the plant organs during early growth, presenting an opportunity to strengthen the root system during early growth by including substances such as humic acid or Biplantol® into the coat which promote preferential partitioning of carbohydrates to the roots. Wheat, in contrast to barley and rye, responded negatively to seed coating, indicating a different sugar metabolism during germination. This and the mechanisms underlying the positive effects of thick coating relative to thin coating on the germination and early seedling growth will be subject of further research.

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References

Anonymous. (2010) The International Seed testing Association (ISTA) International rules for seed testing. Zürichstr. 50 CH-8303 Bassersdorf, Switzerland

Asch, F., Sow, A. and Dingkuhn, M. (1999) Reserve mobilization, dry matter partitioning and specific leaf area in seedlings of African rice cultivars differing in early vigour. Field Crops Research 62: 191-202.

Atiyeh, R.M., Lee, S., Edwards, C.A., Arancon, N.Q. and Metzger, J.D. (2002) The effects of humic acids derived from earthworm-processed organic wastes on plant growth. Bioresource Technology 84: 7-14.

Ayuso, M., Hernandez, T., Garcia, C. and Pascual, J.A. (1996) Stimulation of barley growth and nutrient absorption by humic substances originating from various organic materials. Bioresource Technology 57: 251-257.

Bates, B.C., Kundzewicz, Z.W. and Palutikof, J.P. (2008) Climate change and water. Technical paper of the Intergovernmental Panel on Climate Change, IPCC Secretariat, Geneva, 210pp.

Berdahl, J.D. and Barker, R.E. (1980) Germination and emergence of Russian wildrye seeds coated with hydrophilic materials. Agronomy Journal 72: 1006-1008.

Bioplant Naturverfahren GmbH: Biplantol® . Homeopathy for plants.Carrl-Benz-Str.4 78467 Konstanz,Germany. www.biplanttolusa.com/files/2214902/uploaded/Bioplant_summary.pdf-

Last accessed 15.02.2011

Dehgan, B., Yeager, T.H. and Almira, F.C. (1994) *Photinia* and *Podocarpus* growth response to a hydrophilic polymer-amended medium. Horticultural Science 29: 641-644.

Feldsaaten Freudenberger (2011) Seed coating technology. http://www.mantelsaat.com/startengl.htm - last accessed 18.02.2011.

Fischer, G., van Velthuizen, H., Shah, M., and Nachtergaele, F.O. (2002) Global agro-ecological assessment for agriculture in the 21st century: Methodology and results. pp. 119. Research Report RR-02-02. ISBN 3-7045-0141-7. International Institute for Applied Systems Analysis, Laxenburg, Austria.

Food and Agriculture Organization of the United Nations (FAO) (2003) Impact of climate change on food security and implications for sustainable food production. Committee on world food security. Conference Twenty-ninth Session, 12 to 16 May 2003, Rome.

Food and Agricultural Organization (FAO) (1999) Fermented Cereals: A global perspective. FAO Agricultural Service Bulletin-138. www.fao.org/docrep/x2184E/x2184e03.htm - last accessed 17.02.2011.

Ghebru, M.G., du Toit, E.S and Steyn, J.M. (2007) Water and nutrient retention by aquasoil and Stockosorb polymers. University of Pretoria, Department of plant production and soil science, Pretoria, South Africa.

Gonet, S.S., Dziamski, A. and Gonet, E. (1995) Application of humus preparations from oxyhumolite in crop production. Environment International 22: 559-562.

Gorim, L. and Asch, F. (2010) Effects of hydrophilic coating on the mobilisation of endosperm reserves in seedlings of spring barley (*Hordeum vulgare* L. Maltasia). In: Tielkes, E. (ed.) World food system: A contribution from Europe; book of abstracts; [September 14 - 16, 2010, ETH Zurich, Switzerland] / Tropentag 2010, international research on food security, natural resource management and rural development.

Gorim, L., Asch, F. and Trimborn, M. (2009) Seed coating with hydro-absorbent properties as possible mitigation strategy for unreliable rainfall patterns in early-sown sorghum. In: Tielkes, E. (ed.) Biophysical and socio-economic frame conditions for the sustainable management of natural sources: book of abstracts; [October 6th - 8th, 2009, University of Hamburg, Department of Biology] / Tropentag 2009, international research on food security, natural resource management and rural development.

Hassan, Z.A., Young, S.D., Hepburn, C. and Arizal, R. (1990) An evaluation of urea-rubber matrices as slow-release fertilizer. Fertilizer Research 22: 63-70.

Hlavinka, P., Trnka, M., Semeradova, D., Dubrovsky, M., Zalud, Z. and Mozny, M. (2009) Effect of drought on yield variability of key crops in Czech Republic. Agricultural and Forest Meteorology 149: 431– 442.

Koch, K. (2004) Sucrose metabolism: Regulatory mechanisms and pivotal roles in sugar sensing and plant development. Current Opinion in Plant Biology 7: 235-246.

Loffredo, E., Monaci, L. and Senesi, N. (2005) Humic substances can modulate the allelopathic potential of caffeic, ferulic, and salicylic acids for seedlings of lettuce (*Lactuca sativa* L.) and tomato (*Lycopersicon esculentum* Mill.). Journal of Agricultural and Food Chemistry 53: 9424-9430.

Lukacs, A., Partay, G., Nemeth, T., Csorba, S. and Farkas, C. (2008) Drought stress tolerance of two wheat genotypes. Soil and Water Research 3: 95-104.

Mackowiak, C.L., Grossl, P.R. and Bugbee, B.G. (2001) Beneficial effects of humic acids on micronutrients availability to wheat. Soil Science Society of America Journal 65: 1744-1750.

Olesen, J.E., Trnka, M., Kersebaum, K.C., Skjelvag, A.O., Seguin, B., Peltonen-Sainio, P., Rossi, F., Kozyra, J. and Micale, F. (2011) Review: Impacts and adaptation of European crop production systems to climate change. European Journal of Agronomy 34: 96–112.

Ortiz, R., Sayre, D.K., Govaerts, B., Gupta, R., Subbarao, G.V., Ban, T., Hodson, D., Dixon, J.M, Ortiz-Monasterio, I.J. and Reynolds, M. (2008) Climate change: Can wheat beat the heat? Agriculture, Ecosystems and Environment 126: 46–58.

Peltonen-Sainio, P., Kontturi, M., and Peltonen, J. (2006) Phosphorus seed coating enhancement on early growth and yield components in oat. Agronomy Journal 98: 206-211.

Perata, P., Guglielminetti, L. and Alpi, A. (1997) Mobilization of endosperm reserves in cereal seeds under anoxia. Annals of Botany 79 (SUPPL. A): 49-56.

Powell, A.A. and Matthew, S. (1988) Seed treatment: development and Prospect. Outlook on Agriculture 17: 93-103.

Richardson, M.D. and Hignight, K.W. (2010) Seedling emergence of tall fescue and kentucky bluegrass, as affected by two seed coating techniques. Horticultural Technology 20: 415-417.

Rosenzweig, C., Casassa, G., Karoly, D.J., Imeson, A., Liu, C., Menzel, A., Rawlins, S., Root, T.L., Seguin, B. and Tryjanowski, P. (2007) Assessment of observed changes and responses in natural and managed systems. In Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, pp. 79-131. Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J. and Hanson, C.E. (eds). Cambridge University Press, Cambridge, UK.

Rosenzweig, C. and Hillel, D. (1998) Climate change and the global harvest: Potential effects of the greenhouse effect on agriculture. Oxford University Press. New York. 324 pp.

Sarvas, M., Pavlenda, P. and Takacova, R. (2007) Effects of hydrogel application on survival and growth of pine seedlings in reclamations. Journal of Forest Science 53: 204-209.

Scott, J.M., Jessop, R.S., Steer, R.J. and Mclachlan, G.D. (1987) Effects of nutrients seed coating on the germination of wheat and oats. Fertilizer Research 14: 205-217.

Sharratt, B.S. and Gesch, R.W. (2008) Germination of polymer-coated corn and soybean influenced by tillage and sowing date. Agronomy Journal 100: 585-590.

Silcock, R.G and Smith, F.T. (1982) Seed coating and localised application of phosphate for improving seedling growth of grasses on acid, sandy red earth. Australian Journal of Agricultural Research 33: 785-802.

Soltani, A., Gholipoor, M. and Zeinali, E. (2006) Seed reserve utilisation and seedling growth of wheat as affected by drought and salinity. Environmental and Experimental Botany 55: 195-200.

Tan, K.H. and Nopamornbodi, V. (1979) Effects of different levels of humic acids on nutrient content and growth of corn (*Zea mays* L.). Plant and Soil 51: 283-287.

Tattini, M., Bertoni, P., Landi, A. and Traversim, M.L. (1991) Effects of humic acids on growth and biomass partitioning of container grown olive plants. Acta Horticulturae 294: 75-80.

Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W.H., Simberloff, D. and Swackhamer, D. (2001) Forecasting agriculturally driven global environmental change. Science 292: 281-284.

Türkmen, O., Dursun, A., Turan, M. and Erdinc, C. (2004) Calcium and humic acid affect seed germination, growth, and nutrient content of tomato (*Lycopersicon esculentum* L.) seedlings under saline soil conditions. Acta Agriculturae Scandinavica Section B: Soil and Plant Science 54: 168-174.

Vyn, T.J. and Marua, M. (2001) Polymer seed coatings: Sufficient Risk Reduction for early plant corn. Traditional risks of early planting of uncoated seeds. 56th Annual Corn and Sorghum Research Conference. 1-11. Purdue University.

Willenborg, C.J., Gulden, R.H., Johnson, E.N. and Shirtliffe, S.J. (2004) Germination characteristics of polymer-coated canola (*Brassica napus* L.) seeds subjected to moisture stress at different temperatures. Agronomy Journal 96: 786-791.

3 Seed coating reduces respiration losses and affects sugar metabolism during germination in cereals.

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Abstract

Seed germination and the successful establishment of young seedlings is an important aspect of plant life. Seed germination requires the mobilization of stored reserves and the events involved are not fully understood in all cereals. Seedlings growing from hydro-absorber coated barley, rye and wheat with coat shares greater than 75 % of the average seed have been shown to promote better seedling growth compared to those seedlings growing from uncoated seeds. We investigated how and why these seedlings performed better by analyzing the proportion of reserves mobilized for growth and respiration as well as how sucrose was cleaved in seeds in the presence of hydro-absorber coats. We found that mobilization efficiency was higher resulting in higher biomass in these cereals when they were coated and that the seedling of these cereals cleaved sucrose in different ways. Further investigation needs to be carried out on the enzymes operating in these cereals, individually under coated conditions.

Keywords: Cereals, mobilization efficiency, sucrose metabolism

3.1 Introduction

Viable seeds are the bases of all crop production (TeKrony 2006). Seed research has concentrated on providing technology that sustains or increases the viability and health, successful germination, and homogenous emergence of seeds in different production systems and under a range of constraints (Ehsanfar and Modarres-Sanavy 2005 and Taylor *et al*. 1998). Coating seeds with various substances tries to improve seed to cope with various constraints such as water and nutrient availability, seed dormancy, or sub-optimal temperatures. Seeds have been coated with different substances such as nutrients (Karanam and Vadez 2010; Masuaskas *et al*. 2008; Scott 1998 and Silcock and Smith 1982), hormones (Powell and Mathew 1988), temperature activated coatings (Gesch *et al*. 2012; Vyn and Marua 2001 and Willenborg *et al*. 2004) as well as humic acids, the hydro-absorber (Stockosorb®) and plant fortifiers such as Biplantol® (Gorim and Asch 2012). Particularly, hydro-absorbers when included in seed coats are thought to increase the water availability to seedlings and thus promote germination and early seedling growth. Whereas seed coats dotted with hydro-absorbers have been shown to successfully increase seed performance in grasses (Leinauer *et al*. 2010 and Serena *et al*. 2012), cereals seed coats containing hydro-absorbers often result in low germination rates (Gorim and Asch 2012). We previously reported that in general cereal species responded differently to coats and that the coat thickness had a strong effect on germination rates and biomass partitioning during early seedling growth and that seed coats improved seedling performance when the coat made up at least 75% of the total grain even when germination rates were low (Gorim and Asch 2012). The differences found between the cereal species, as well as the differences between uncoated and thickly coated seeds raise the question if coating affected the early starch breakdown and thus seedling growth via changes in the sugar availability during remobilization of grain reserves.

Cereal seeds are composed of a large, starch containing endosperm surrounded by a protein-rich and physiologically active aleurone layer, and the living tissue of the embryo which is in direct contact with the endosperm via the scutellum that replaces the aleurone layer in this position. For cereal seeds to germinate, the stored starch in the endosperm has to be broken down into hexoses. In contrast to the embryo, the endosperm constitutes dead tissue at the time of germination so that enzymes that break down starch need to be released into the endosperm either from the aleurone layer or from the scutellum (Zeeman *et al*. 2010). Consequently, an intense synthesis of α-amylase in the aleurone and scutellum of germinating cereal seeds has been observed that were excreted into the endosperm (Fincher 1989).

Starch is eventually broken down into glucose and maltose and sucrose is synthesized in the endosperm, the scutellum or both from mobilized fractions of the starchy endosperm (Fincher 1989). Seed reserves can be thus channeled into growth either directly via hexoses derived from starch cleavage and diffusive transport into surrounding tissues (Matsukura *et al*. 2000) or indirectly through sucrose synthesis, active transport and subsequent cleavage at the site of physiological activity. Sucrose can either be formed from mobilized starch involving the enzymes sucrose synthase and sucrose phosphate synthase (Aoki *et al*. 2006 and Kennedy *et al*. 1992), it can already be present in the endosperm as disaccharide storage product as shown for barley by Guglielminetti *et al*. 1999 and for wheat and rye by Halford *et al*., 2011 or it can be secreted into the endosperm from catabolic degradation of lipids in the aleurone layer (as shown for wheat by Chrispeels *et al*. 1973) making thus sucrose the most abundant sugar in the endosperm during early germination (Aoki *et al*. 2006).

The current study focuses on the effects of seed coats containing hydro-absorber with a share of coat in the total grain above 75% on early seedling growth and respiration in barley, rye, and wheat as related to sugar mobilization.

3.2 Materials and methods

3.2.1 Plant material and treatments

Seeds of spring barley (*Hordeum vulgare* L., cv. Maltasia), winter rye (*Secale cereal* L., cv. Jobaro), and spring wheat (*Triticum aestivum* L., cv. Thasas) were obtained from Freudenberger Feldsaaten GmbH and used in all experiments. Seeds of all 3 cereal species were either uncoated or coated, with a seed coat developed by Freudenberger Feldsaaten GmbH containing a specific amount of hydro-absorber (Stockosorb®). The term “coated” refers to grains with a coat share greater than 75 % whereas the term “uncoated” refers to the original seed.

3.2.2 Reserve partition in cereals during growth

Uncoated and coated grains were individually weighed and placed on filter paper moistened with 12 mL of distilled water (Ecolab-Bogen-Filterpapier; Neolab Heidelberg, Germany) in PET plates of 19.5 x 19.5 cm, their position was noted, and they were germinated in a growth chamber (Percival Scientific Inc., Perry, IA, USA) at 25 °C with a 12-h photoperiod. Grains were spaced 3 x 2.5 cm in the plates replicated three times within each experiment, which was each repeated three times. To ensure that the filter paper remained moist, 10 mL of distilled water was added into each plate daily. Twenty five plants sampled daily over a 2 weeks period, were divided into root, shoot, and remaining grain. Two days after soaking, seeds were assessed for germination. When a tiny radicle was observed seeds were counted as germinating and samplings were conducted every 2 days from this day. To each plate was added 15 mL Wuxal® nutrient solution 9 days after soaking (DAS). Coatings were washed off from coated grains and the remaining kernel, root, and shoot oven dried at 70°C for 48 h for the determination of their dry weights. Kernel and seedling weights were observed for 14 DAS. A regression was fitted for seedling dry weight development and kernel weight over time with SigmaPlot 10.0, Systat Software GmbH. Germination, growth respiration, and early seedling growth was analyzed following the procedure described by Asch *et al*. (1999). Two regressions were fitted to the data: Regression I describes the linearly decreasing portion in kernel weight over time while regression II describes the linear decrease of the seedling dry matter over time. The efficiency of reserve mobilization was calculated as one minus the ratio of the slope of regression II divided by the slope of regression I. The weight lost on a dry weight basis (∂), in milligrams, as reserves were mobilized from the grain over time was obtained by subtraction of dry weights between any 2 points in time. The additional dry matter (∂_1) produced as time progressed in the roots, shoots and total dry matter was also determined.

3.2.3 Preparation of plant materials for sugars analysis

Grains, from both coated and uncoated seeds, were taken out every 2 days for 14 days after they were placed on the moist filter paper for sample collection. The coats were washed off the coated seeds, and for both coated and uncoated seeds, embryonic material was quickly excised on ice with a stainless steel razor blade, and freeze dried (Freeze dryer, LYOVAC GT2-33520, Riedstadt, Germany) in 2 mL Eppendorf vials. Every 2 days after germination meristematic tissue from roots (the apical 4 cm of the root below the root hair zone) and shoots (the basal 1 cm of the shoot) were harvested, freeze dried in 2 mL Eppendorf vials and later analyzed separately.

3.2.4 Extraction and quantification of sugars

Ten milligrams of freeze dried roots and shoot bases per replicate were ground in 'FastPrep Lysing matrix A' tubes at 6.0 MS for 40 seconds in the FastPrep® - 24 homogenizer. 500 µL 80% ethanol was added, the tubes vortex and heated at 60 °C for 30 minutes. Each tube was centrifuged for 10 minutes at 13,000g and the supernatant transferred to another Eppendorf-vial. The process, from addition of ethanol to centrifugation was repeated twice. The ethanol extract was evaporated to dryness in a vacuum centrifuge (Eppendorf Concentrator 5301) at 45 °C for 2.5 h and re-dissolved in 900 µL water by shaking overnight.

Sugars were assayed as described by Andersen *et al*. (2002). For glucose, 100 µL were assayed in a total volume of 200 µL water. The dye, glucose oxidase/peroxidase/ABTS-solution (400 µL) was added and samples incubated in a water bath at 37°C for 30 minutes.

For sucrose plus glucose, 50 µL were assayed in a total volume of 300 µL in NaAc-buffer containing 50 mM Na-acetate and 15 mM magnesium chloride (pH 4.6). Invertase solution, 2 µL and the dye were added. Samples were read at 418 nm in a spectrophotometer (Beckmann, Type UV-Du640, USA).

A standard curve was established by dissolving 1.50 mg glucose in buffer solution as maximum concentration. The standard was diluted to derive a linear calibration curve with an $r^2 > 0.99$.

3.2.4.1 Calculation of the amount of sugars

From the linear standard curve, the amount of sugars in each sample measured per milligram dry matter or milligram reserves remobilized was calculated. For example: The amount of glucose per milligram sample (mg/mg) in an unknown sample

$$x = \frac{[0.300\text{ml}].\left[\text{Y1}\left(\frac{\text{mg}}{\text{ml}}\right)\right].[0.900\text{ml}]}{[0.05\text{ml}].[\text{Zmg}]}$$

Where:

x is the amount of sugar in mg/mg dry weight plant material

Y1 (mg/ml) is the concentration of glucose obtained from the standard curve

0.300ml. [Y1 (mg/ml)] is the amount of glucose in 300 µL assayed (mg)

[0.300ml. [Y1 (mg/ml)].[(0.900/0.05 ml)] is the amount of glucose in the total (900µL) samples

Z mg can either be weight lost (∂) from grain or increased in dry matter (∂_1)

Statistical Analysis

Comparisons in mobilization efficiency, onset of autotrophy and compensation time between coated and uncoated treatments for each cereal was done with the aid of the student t-test at alpha equals 5%. Comparison between the rate at which seed reserves were mobilized in the kernel over time (regression I) in both coated and uncoated seeds as well as the linear decrease in the total dry matter (regression II) between coated and uncoated seeds were assessed by checking for the interaction term employing Proc GLM procedure in SAS.

3.3 Results

3.3.1 Mobilization of grain reserves and seedling growth over time

The average initial grain weight used in this kinetic study was obtained from a 1000-g grain distribution count (Gorim and Asch, 2012). The average weight for the uncoated barley, rye, and wheat grains was 46.2 mg, 44.3 mg and 42.0 mg respectively. The coated and uncoated seeds showed germination and early seedling growth patterns similar to those reported by Asch *et al*. (1999). However, the current study contrasted that of rice wherein the onset of photo autotrophy coincided with the end of mobilization of endosperm reserves. The initial dry weight (DW) loss to respiration was compensated earlier in coated seeds compared to the uncoated seeds (Fig. 1).

The kernel DW decreased linearly until 8 - 10 DAS in both coated and uncoated seeds in all species, after which it leveled off at 11% and 12 % for coated and uncoated barley seeds respectively, 16% and 10% for coated and uncoated rye seeds respectively and 11% and 8% for coated and uncoated wheat seeds respectively, of the initial kernel DW (Fig.1).

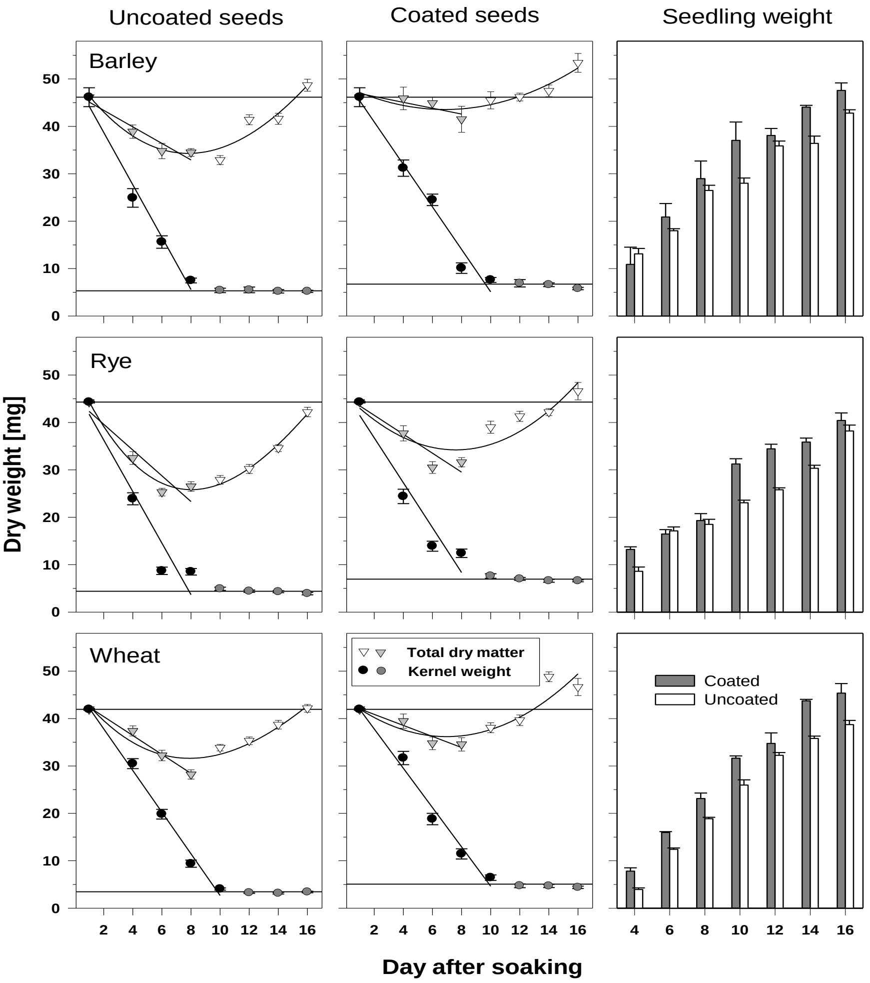

Fig. 1 Reserve mobilization, early seedling growth, dry matter produced during early growth from coated and uncoated barley, rye and wheat seeds. Regression I (plots with black dots) = decreasing kernel weight over time; Regression II (plots with gray triangles) = decrease of total seedling dry matter (roots, shoots and remaining kernel) over time. The efficiency of reserve mobilization was calculated as one minus the ratio of the slope of regression II over the slope of regression I. The linear regression analysis was used to determine the reserve mobilization, onset of autotrophy and mobilization efficiency. White triangles = total dry matter after the onset of photo-autotrophy; dark gray dots = remaining kernel weight after the end of reserve mobilization. Seedling weight = sum of dry weights of roots and shoots. Error bars: Standard error of mean.

Consequently, between 88-89 %, 84-90 % and 89-92 % of coated and uncoated barley, rye and wheat kernel DW respectively was mobilized during germination and relocated to the seedling or used in respiration. There was variability in the mobilized DM fractions which depended on the presence of the coat and species (Fig 1).

The mobilized fractions were similar between coated and uncoated grains as evident in the differences of kernel DW loss for each species, but the rate at which reserves were mobilized was significantly different.

3.3.2 Effect of seed coating on mobilization efficiency and seedling growth

Coated barley and wheat seeds had higher mobilization efficiencies as compared to the uncoated seeds and the onset of photo autotrophy was also found to be earlier in coated grains in all species in comparison to the uncoated seed (Table 1). These higher mobilization efficiencies imply that in coated seeds larger proportions of endosperm reserves were directly converted into growth and, consequently, respiration was lower. This is evident in the seedling DW that was higher for coated seeds of all species compared to the uncoated seeds over the entire growth period (Fig.1). In turn, the contribution of seedling photosynthesis to growth in order to compensate losses to respiration was higher in the uncoated seeds compared to the coated; 11.9 to 2.6 mg in barley, 18.5 to 10.1 mg in rye and 10.3 to 5.8 mg dry matter in wheat. The high mobilization efficiency observed in coated compared to uncoated seeds resulting in higher growth rates, smaller respiration losses, and earlier compensation of respiration losses during germination implies a difference in the sugar metabolic pathway during seedling growth from coated in contrast to uncoated seeds.

Table 1

Linear regressions used to determine the mobilization efficiency of grain reserves, the onset of autotrophy, and the time to reach initial kernel weight of barley, rye, and wheat seeds that were coated with seed coating containing the hydro-absorber Stockosorb compared to uncoated seed. Regression I refers to kernel weight loss over time in Figure 1 and Regression II refers to initial weight losses due to growth respiration of the seedling in Figure 1. Letters indicate significant difference between coated and uncoated seed at $p \leq 0.05$ with the absence of letters indicating no significant difference.

Cereals	Treatments	Regression I (mg/d)	Regression II (mg/d)	Mobilization efficiency (mg/mg*)	Onset of Autotrophy (days)	Compensation time (days)
Barley	Coated	$49.9-4.5x$	$47.6-0.6x$	0.86 (±0.03)^{a}	7.0 (±0.2)^{b}	11.9 (±0.4)^{b}
	Uncoated	$49.8-5.5x$	$46.9-1.7x$	0.68 (±0.03)^{b}	7.9 (±0.2)^{a}	15.2 (±0.4)^{a}
Rye	Coated	$46.3-4.7x$	$45.5-2.0x$	0.58 (±0.03)	7.6 (±0.2)	14.7 (±0.5)^{a}
	Uncoated	$47.2-5.4x$	$45.1-2.7x$	0.50 (±0.03)	7.9 (±0.2)	16.8 (±0.2)^{b}
Wheat	Coated	$46.1-4.2x$	$43.2-1.2x$	0.72 (±0.04)^{a}	7.0 (±0.2)^{a}	12.8 (±0.4)^{b}
	Uncoated	$46.6-4.4x$	$44.4-2.0x$	0.55 (±0.01)^{b}	7.9 (±0.2)^{b}	15.7 (±0.2)^{a}

Linear regression analyses were performed with SigmaPlot 10.0. For details, see Fig. 1.

3.3.3 Effects of seed coating on seedling sugar availability

A strong indicator for the effects of seed coating on transfer of mobilized endosperm reserves into seedling growth is the amount of available sugars in the growing seedling. Small losses of mobilized endosperm reserves to respiration as observed in seedlings from coated seeds in barley and wheat (Fig. 1) should result in lower glucose levels in growing seedlings as the glucose is more efficiently metabolized into tissue structures. In young seedlings from coated seeds of barley and wheat, glucose levels were significantly lower than in seedlings from uncoated seeds whereas in rye they tended to be higher with the pattern not as clear (Fig. 2 a-c). The amount of sucrose (Fig. 2d-f) in young seedlings was in general inversely proportional to that of glucose. However, the three species showed different patterns related to the effect of seed coating. Whereas sucrose levels were significantly higher in seedlings from coated seeds in barley from 6 DAS, no clear pattern emerged in rye, and in wheat sucrose level in seedlings from uncoated seeds tended to be higher than those in seedlings from coated seeds. These patterns were supported by the ratio of glucose over sucrose (Fig. 2 g-i). The non-regular patterns for glucose and sucrose found in rye seeds indicate that seed coating was not the decisive factor influencing sugar metabolism in rye, whereas the general patterns in barley and wheat indicate a clear change in sugar metabolism due to seed coating. These results relate well to the differences in mobilization efficiency between the species and between coated and uncoated seeds as shown in Table 1.

3.3.4 Relationship between sugar mobilization and early seedling growth

In general, efficiency in mobilization of endosperm reserves is achieved by breaking down starch into sucrose and then sucrose into hexoses. Figure 3 a-c shows that in all three species, seedlings from coated seeds grew more vigorously per unit grain dry matter mobilized than seedlings from uncoated seeds. The dotted line indicates the theoretical 100% conversion of grain dry matter to seedling growth.

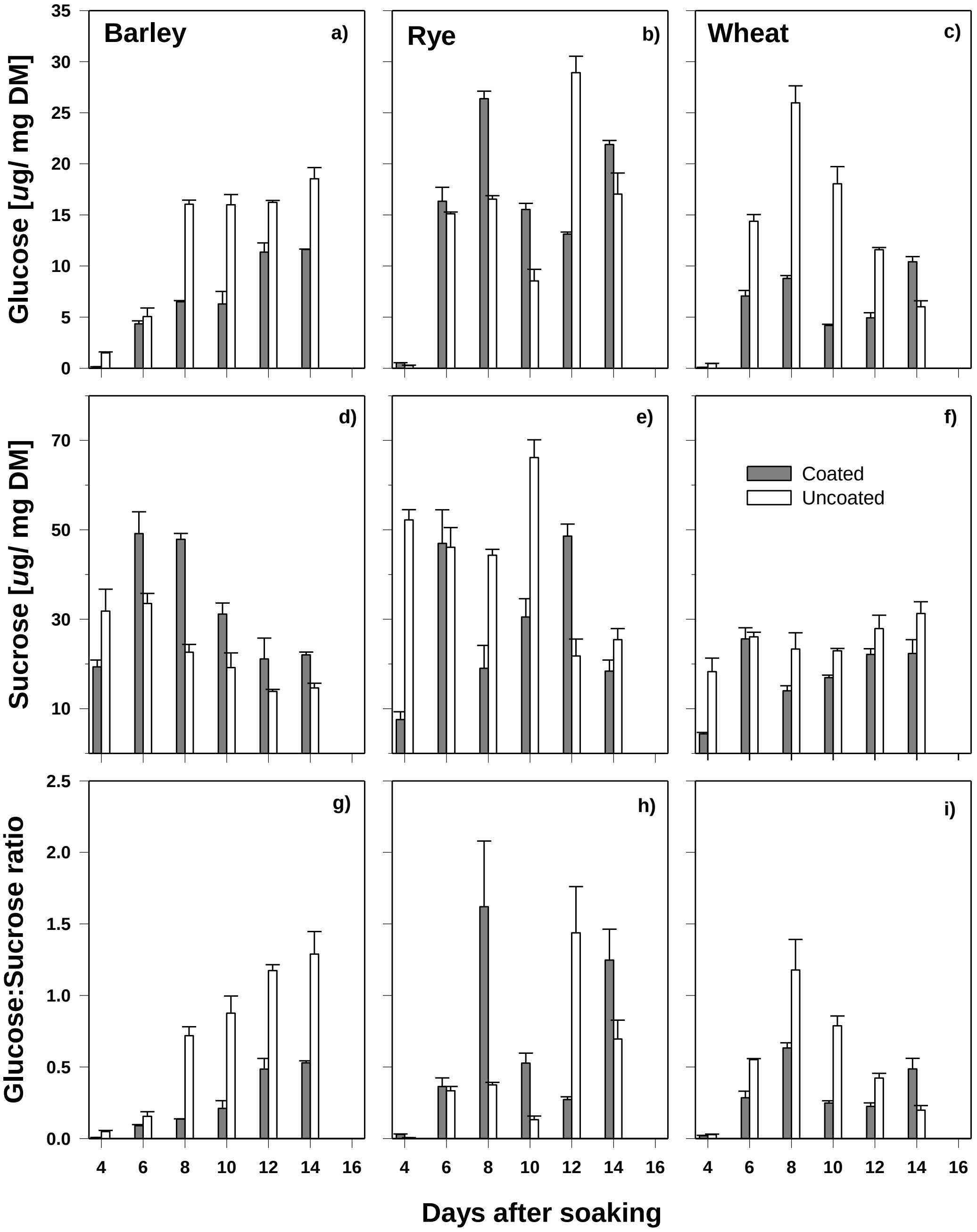

Fig. 2 Glucose and sucrose concentration as well as the glucose : sucrose ratio during the first 14 days of early seedling growth in the seedlings grown from coated and uncoated seed of barley, rye, and wheat. Error bars: Standard error of mean.

Rye, in contrast to barley and wheat, had the lowest conversion rate from dry matter mobilized to seedling growth for both treatments. The difference in mobilization efficiency and in the conversion rate indicates differences in the effectiveness of starch and, eventually, sucrose breakdown. Since sucrose after being synthesized for example in the scutellum is actively transported into the embryonic tissue and cleaved there into sucrose and fructose, we have measured both sucrose and glucose concentrations in the growing seedling and used the cumulated amounts of both sugars as an indicator of the amount available to the seedling during germination and early seedling growth (Fig. 3 d-f and g-i). In barley, the sucrose available in the seedling resulted in the same seedling growth rate independent of the seed coating, indicating a similarly efficient starch breakdown and subsequent conversion into sucrose for both treatments (Fig. 3d). In contrast for rye and wheat, seedlings from coated seeds accumulated more dry matter per unit sucrose available to the seedling than seedlings from uncoated seeds. When their slopes were compared, uncoated seeds from rye produced only 25% of the seedling dry matter produced from uncoated seeds in wheat per unit sucrose available to the seedling, indicating a highly inefficient breakdown of sucrose in the embryonic tissue (Fig. 3e, f). Embryonic sucrose is eventually cleaved into glucose and fructose and the resultant glucose metabolized during germination under aerobic conditions in its phosphorylated form into new metabolic compounds. Thus, sucrose forms the basis for seedling growth and the effective transfer of hexoses into growth determines the growth rate of the seedling. Figure 3 g-i shows the relationship between glucose available to the seedling during the mobilization of grain reserves and seedling growth. In barley, the amount of glucose required to produce 30mg of seedling dry matter quadruples in seedlings from uncoated seed as compared to seedlings from coated grains (Fig. 3g). In wheat, about three times the amount of glucose is required to produce 30mg of seedling dry matter from uncoated seeds when compared with coated seeds and about 50% more than from uncoated seeds in barley (Fig. 3i). In rye, no difference was observed in the dry matter produced from glucose available to the seedlings between seeds of the two treatments (Fig. 3h).

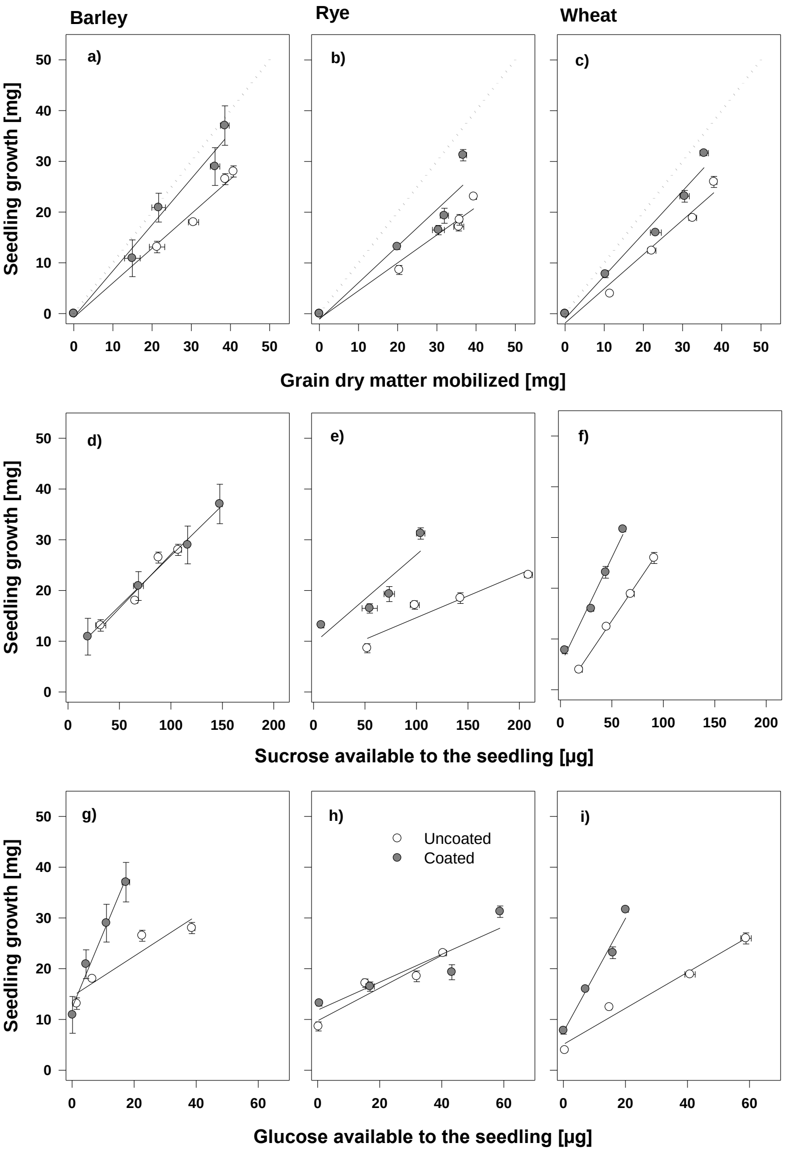

Fig. 3 All data refers to seedlings grown from uncoated and coated seed from barley, rye, and wheat. Upper row of sub graphs: Dry matter mobilized from grain reserves as related to early seedling growth. Middle row of sub graphs: Measured amounts of sucrose cumulatively made available to the seedling during the first 14 days of early seedling growth as related to seedling growth during the first 14 days. Bottom row of sub graphs: measured cumulative glucose made available to the seedling during the first 14 days of early seedling growth as related to seedling growth during the first 14 days. Error bars: Standard error of mean.

Except for species specific conversion factors, it can be expected that a given amount of glucose is needed to produce a given amount of dry matter, thus, either there is another source of energy available for growth in seedlings from coated grains in barley and wheat, or some of the glucose available to seedlings from uncoated grains is lost in the growth process, probably to respiration.

3.4 Discussion

3.4.1 Effects of coating on reserve mobilization and seedling growth

As previously shown, germination success of seeds enveloped in coating depends on the share of the coating material in the total grain. Thick coats containing Stockosorb and having a share larger than 75% of the total grain reduced germination to various extends in the three cereal species tested here (Gorim and Asch 2012). A thick coat is likely to change the water supply and water uptake of the seed as compared to the uncoated seed as the hydro-absorber; Stockosorb can retain water at about 400 times its weight. This effect most likely affects the germination process and the early seedling growth but almost no information on these effects is available in literature to date. In all three cereal species, growth vigor of the early seedling was increased in seedlings from coated grains. Species differed in this respect as shown in the differences in the slopes shown in Figure 3 a-c. Most striking differences were found in mobilization efficiency. In general, barley had the highest mobilization efficiencies for both coated and uncoated seeds as compared to the other two species. In coated seeds, mobilization efficiency increased in barley by factor 1.26, in rye by 1.16, and in wheat by 1.30 when compared to the uncoated seed (Table 1), indicating that the coating effect on mobilization efficiency in barley and wheat was similar despite wheat being in general less effective in mobilizing grain reserves. Little attention has been paid to the efficiency of mobilization of grain reserves in cereals so far.

Scattered reports are available for wheat and rice only but not for barley or rye. Labusch *et al*. (1989) for winter wheat and Soltani *et al*. (2006) for spring wheat reported genotype depending mobilization efficiencies 0.52 and 0.7 which are in the range of the values reported here. All those values were obtained from uncoated grains germinated in petri dishes for 5-7 days and did not vary with the different treatments applied. A strong dependency on temperature and genotype in the efficiency of grain reserve mobilization in wheat was observed by Hasan *et al*. (2004) with optimum temperatures for maximum efficiency at 25°C which corresponds to the temperature used in all experiments reported here. Mobilization efficiency varies not only between varieties of the same cereal as reported in rice (Asch *et al*. 1999) but is also influenced by the cereal species, as we have shown here. In addition, coating strongly reduced the amount of mobilized grain reserves invested in respiration. The proportion of mobilized reserves channeled into growth is determined in the seed by sugar sensors, enzymes, and other factors such as oxygen availability (Guglielminetti *et al*. 1999; Koch 2004 and Perata *et al*. 1992) and efficiency of both the transport of sucrose synthesized from hexoses after the breakdown of starch in the endosperm and its subsequent cleavage into glucose and fructose at the site of metabolism.

3.4.2 Effects of coating on sugar metabolism and seedling growth

Sucrose can be cleaved by two enzymes, namely invertases and sucrose synthase. The relative activity of these two enzymes has been proposed as key determinant of carbon partitioning and seems to play a role in the control of development (Halford *et al*. 2011). Invertases cleave sucrose into glucose and fructose and in order to be physiologically active ATP depending hexokinases are needed to phosphorylate these hexoses. Sucrose synthase, on the other hand, cleaves sucrose into uridine diphosphate glucose and fructose a process that is not ATP dependent (Koch 2004 and Perata *et al*. 1997). The activity of either enzyme depends on the oxygen level of the tissue they are operating in. In low oxygen environments, invertases are said to be inhibited (Zeng *et al*. 1999) whereas under the same conditions sucrose synthase encoding genes and sucrose synthase activity are up-regulated (Zeng *et al*. 1998). Thus, the pathway in which sucrose is split predominantly has a significant effect on the overall ATP produced at the end of glycolysis. The sucrose synthase pathway is said to be energy efficient, conserving at least one adenosine triphosphate molecule, promotes storage (Koch 2004 and Sung *et al*. 1988) and synthesis of cell wall polysaccharides such as cellulose, pectin, and arabinoxylans (Halford *et al*. 2011). This means that germinating seeds employing this pathway are likely to channel their stored reserves directly into growth rather than lose them to respiration. The three species in our study showed strongly different patterns in the amount of sucrose available to seedling growth for both coated and uncoated seeds. For both coated and uncoated seeds in barley, sucrose available to the seedling and seedling growth was strongly linearly correlated with the seedlings growing from coated seeds achieving better growth and mobilization larger amount of sucrose in the seedling (Fig. 3d). This indicates a normal starch breakdown in the endosperm via amylases channeled into sucrose synthesis and then growth. However, when the hexose compound, glucose from sucrose is considered, seedlings from coated seeds seemed to grow with only one third of the glucose required by the seedlings from the uncoated seeds (Fig 3g). This indicates a strong switch from the invertase based cleavage of sucrose to the sucrose synthase based cleavage of sucrose in the embryo also since the assay employed here to detect glucose does not detect UDP-glucose. As stated above, a low oxygen environment promotes the activity of sucrose synthase, thus, these data suggest a change in oxygen availability within the embryo induced by the coating.

In rye, an inverted pattern was found. Seedlings from coated seeds seemed to grow with only about 40% of the sucrose mobilized for growth to seedlings from uncoated seeds (Fig. 3e); Indicating another source of sugars for growth, maybe fructose from sucrose mobilized via fructan catalyzed by fructosyl transferases may play major role here since rye has superior freezing tolerance and greater capacity for carbohydrate storage (Halford *et al*. 2011). Consequently, the slope for glucose available to the seedling as related to seedling growth did not differ between the two treatments, with the seedlings from coated seeds still showing increased growth as compared to the seedlings from uncoated grains (Fig. 3h). This suggests a direct transfer of glucose from the endosperm to the embryo without involving endosperm based sucrose synthesis in the rye seedlings growing from coated seeds as suggested by Perata *et al*. (1997) for anoxic conditions and thus an effect of the coating entirely different from that in barley.

In wheat, again a different pattern was found that differed strongly from barley for sucrose availability to the embryo and differed strongly from rye with regard to glucose available in the embryo (Fig. 2, Fig. 3). Sucrose synthesis in the endosperm or scutellum was strongly linked to seedling growth in both seedlings from coated and uncoated seeds. As compared to rye, wheat seedlings grown from uncoated seeds required only about one third of the sucrose to produce similar amounts of dry matter (Fig. 3f) which relates well to the differences in respiratory losses during early seedling growth between the two species (Fig. 1), but is not reflected in the mobilization efficiency in uncoated seedlings from both species (Tab. 1). In contrast, glucose available in the seedling was similar to the two other species for the seedlings grown from uncoated seed in wheat (Fig. 3g-i), whereas when growing from coated seed, glucose available in the seedling as related to seedling growth was similar to barley and about twice as effective as in rye. This indicates an early starch and sugar metabolism using both the direct import of glucose and an increased import of UDP glucose into the seedling in wheat seedlings grown from coated seed.

3.4.3 Conclusion and outlook

We have shown here, that during the first 2 weeks of germination and early seedling growth, seedlings from barley, rye and wheat differed strongly in their efficiency of grain reserve mobilization, their sucrose and their glucose embryo concentrations and their effectiveness to translate sucrose or glucose accumulated from the endosperm into seedling growth when the seedlings were grown from seed coated with a seed coat containing Stockosorb as a main component. Following the definition of Nonogaki *et al*. (2010) of the different phases of germination, we studied here mainly effects of phase III of germination (post-germination phase) that had their origin during the first two phases of germination. The manner in which sucrose and glucose availability and transfer into growth differ among the species indicates a strong effect on the enzymatic break down of starch and sucrose during phase II of germination. The changes observed indicate possible strong differences either in the water or in the oxygen availability during early germination influencing translation or degradation of seed-stored mRNA. It has been shown that for example in barley grain embryos more than 12,000 mRNAs are stored including many related to seed reserve mobilization. Within the first 24h hours of germination, transcription of genes encoding cell wall bound and vacuolar invertases, sucrose synthases, and sucrose phosphate synthase are activated in the aleurone and embryo (Sreenivasalu *et al*. 2008). It has also been shown that this transcriptome undergo dramatic changes within the first hours of imbibition and germination (Howell *et al*. 2009) of which many are related to the degradation of stored mRNAs (Weitbrecht *et al*. 2011).

We conclude, therefore, that the first few hours and days of the germination process are decisive for the effectiveness of grain reserve mobilization and also which enzymatic pathway will be employed for sugar mobilization under given conditions of water and oxygen availability as defined by the presence of a seed coat. Seed imbibition rate and seed oxygen profiles as affected by seed coating during the first 48 h of germination will be the focus of future research in order to elucidate which metabolic processes are responsible for the reduction of respiration losses during grain reserve mobilization in germinating barley, rye and wheat coated seed compared to uncoated ones.

References

Andersen, N.M., Asch, F., Wu, Y., Jensen, R.C., Naested, H., Mogensen, O.V. and Koch, E.K: 2002. Soluble invertase expression is an early target of drought stress during the critical, abortion-sensitive phase of young ovary development in maize. Plant Physiology 130: 591-604.

Aoki, N., Scofield, G.N., Wang, X.D., Offler, C.E., Patrick, J.W.and Furbank, R.T: 2006: Pathway of sugar transport in germinating wheat seeds. Plant Physiology 141: 1255-1263.

Asch, F., Sow, A. and Dingkuhn, M. 1999: Reserve mobilization, dry matter partitioning and specific leaf area in seedlings of African rice cultivars differing in early vigour. Field Crops Research 62: 191-202.

Chrispeels, M.J., Tenner, A.J. and Johnson, K.D. 1973: Synthesis and release of sucrose by the aleurone layer of barley: Regulation by gibberelic acid. Planta 113: 35-46.

Ehsanfar, S. and Modarres-Sanavy, S.A. 2005: Crop protection by seed coating. Communications in Agricultural and Applied Biological Sciences 70: 225-229.

Fincher, G.B. 1989: Molecular and cellular biology associated with endosperm mobilization in germinating cereal grains. Annual Review of Plant Physiology and Plant Molecular Biology 40: 305-346.

Gorim, L. and Asch, F. 2012: Effects of composition and share of seed coatings on the mobilization efficiency of cereal seeds during germination. Journal of Agronomy and Crop Science 198: 81-91.

Gesch, W.R., Archer, W.D. and Spokas, K. 2012: Can using polymer-coated seed reduce the risk of poor soybean emergence in no-tillage soil? Field Crops Research 125: 109-116.

Guglielminetti, L., Loreti, L., Perata, P. and Alpi, A.1999: Sucrose synthesis in cereal grains under oxygen deprivation. Journal of plant Research 112: 353-359.

Halford, N.G., Curtis, T.Y., Muttucumaru, N., Postles, J. and Mottram, D.S. 2011: Review Article: Sugars in crop plants. Annals of Applied Biology 158: 1-25

Hasan, M.A., Ahmed, J.U., Hossain, T., Hossain, M.M. and Ullah, M.A. 2004: Germination characters and seed reserve mobilization during germination of different wheat genotypes under variable temperature regimes. Journal of the National Science Foundation of Sri Lanka 32: 97-107.

Howell, K.A., Narsai, R., Carroll, A., Ivanova, A., Lohse, M., Usadel, B., Millar, A.H. and Whelan, J. 2009: Mapping metabolic and transcript temporal switches during germination in rice highlights specific transcription factors and the role of RNA instability in the germination process. Plant Physiology 149: 961–980.

Karanam, V.P. and Vadez, V. 2010: Phosphorus coating on pearl millet seed in low P alfisol improves plant establishment and increases stover more than seed yield. Experimental agriculture 46(4): 457-469

Kennedy, R.A., Rumpho, M.E. and Fox, T.C.1992: Anaerobic metabolism in plants. Plant Physiology 100 (1): 1-6.

Koch, K. 2004: Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Current Opinion in Plant Biology 7: 235-246

Labusch, U., Schnyder, H. and Kuhbauch, W.1989: Use and partitioning of grain reserves and products of current synthesis during the transition from heterotrophic to autotrophic assimilate supply in wheat seedlings. Mitteilungen der Gesellschaft für Pfanzenbauwissenschaften 2: 77-80.

Leinauer, B., Serena, M., and Singh, D. 2010: Seed coating and seeding rate effects on turfgrass germination and establishment. HortTechnology 20: 179-185.

Masuaskas, S.V., Masuaskiene, A., Repsiene, R., Skuodiene, R., Braziene, Z. and Peltonen, J. 2008: Phosphorus seed coating as starter fertilization for spring barley. Acta Agriculturae Scandinavica Section B: Soil and Plant Sciences 58: 124-131.

Nonogaki, H., Bassel, G.W. and Bewley, J.D. 2010: Germination-still a mystery. Plant Science 179: 574-581.

Perata, P., Pozueta-Romero, J., Akazawa, T. and Yamaguchi, J. 1992: Effect of anoxia on starch breakdown in rice and wheat seeds. Planta 188: 611-618.

Perata, P., Guglielminetti, L. and Alpi, A. 1996: Anaerobic carbohydrate metabolism in wheat and barley, two anoxia-intolerant cereal seeds. Journal of Experimental Botany 47: 999-1006.

Perata, P., Guglielminetti, L., and Alpi, A. 1997: Mobilization of endosperm reserves in cereal seeds under anoxia. Annals of Botany 79: 49-56.

Powell, A.A., and Matthew, S. 1988: Seed treatment: development and prospect. Outlook on Agriculture 17: 93–103.

Serena, M., Leinauer, B., Sallenave, R., Schiavon, M., and Maier, B. 2012: Turfgrass establishment from polymer-coated seed under saline irrigation. Hort Science 47: 1789-1794.

Scott, J.M. 1998: Delivering fertilizers through seed coatings. Journal of Crop Production 1: 197-220.

Silcock, R., and Smith, F. 1982: Seed coating and localized application of phosphate for improving seedling growth of grasses on acid, sandy red earth. Australian Journal Agricultural Research 33: 785–802.

Soltani, A., Gholipoor, M. and Zeinali, E. 2006: Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. Environmental and Experimental Botany 55: 195-200.

Sreenivasulu, N., Usadel, B., Winter, A., Radchuk, V., Scholz, U., Stein, N., Weschke, W., Strickert, M., Close, T.J., Stitt, M., Graner, A. and Wobus, U. 2008: Barley grain maturation and germination: Metabolic pathway and regulatory network commonalities and differences highlighted by new MapMan/PageMan profiling tools. Plant Physiology 146 (4) 1738-1758.

Sung, S.S., Xu, D and Black, C.C. 1988: Identification of actively filling sucrose sinks. Plant Physiology 89: 1117-1121.

Taylor, A.G., Allen, P.S., Bennett, M.A., Bradford, K.J., Burris, J.S., and Misra, M.K. 1998: Seed enhancements. Seed Science Research 8: 245-256.

TeKrony, D.M. 2006: Seeds: The delivery system for crop science. Crop Science 46: 2263-2269.

Vyn, T.J. and Marua, M. 2001: Polymer seed coatings: sufficient risk reduction for early plant corn. Traditional risks of early planting of uncoated seeds. 56th Annual Corn and Sorghum Research Conference. 1–11. Purdue University.

Weitbrecht, K., Müller, K. and Leubner-Metzger, G. 2011: First off the mark: Early seed germination. Journal of Experimental Botany 62: 3289-3309.

Willenborg, C.J., Gulden, R.H., Johnson, E.N., and Shirtliffe, S.J. 2004: Germination characteristics of polymer-coated canola (Brassica napus L.) seeds subjected to moisture stress at different temperatures. Agronomy Journal 96: 786–791.

Zeeman, S.C., Kossmann, J. and Smith, A.M. 2010: Starch: Its metabolism, evolution, and biotechnological modification in plants. Annual Review of Plant Biology 61: 209-234.

Zeng, Y., Wu, Y., Avigne, T.W. and Koch, E. K. 1998: Differential regulation of sugar-sensitive sucrose synthases by hypoxia and anoxia indicate complementary transcriptional and posttranscriptional responses. Plant Physiology 116: 1573-1583.

Zeng, Y., Wu, Y., Avigne, W.T.and Koch, E. K. 1999: Rapid repression of maize invertases by low oxygen. Invertase/sucrose synthase balance, sugar signaling potential, and seedling survival. Plant Physiology 121: 599-608.

4 Seed coating increases seed moisture uptake and restricts embryonic oxygen availability in germinating cereal seeds.

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Abstract

Seed coating is a technology to improve germination and homogenize stand establishment. Although coating often results in lower germination rates, seeds that do germinate grow more vigorously and show strongly reduced respiratory losses during reserve mobilization. We hypothesize that the higher mobilization efficiency is due to a shift in the enzymatic cleavage of sucrose from invertase to sucrose synthase in the embryonic tissue caused by a reduced oxygen availability induced by over saturation with water caused by the coating during early germination. We investigated the effect of coating on barley, rye and wheat seed imbibition during the first 30 hours after seeds were placed in moisture. For the first time, we profile oxygen in these cereals' embryos and measured sucrose and acid invertase levels as imbibition progressed. We found that seeds within coatings absorbed significantly more moisture than uncoated seeds. Coating resulted in near anoxic oxygen concentrations in the developing embryonic tissues in all three species. In barley, clearly sucrose was not cleaved via the invertase pathway anymore, despite the fact that invertase activity levels in coated seeds were increased. In rye and wheat, clear evidence from the sugar availability in the embryo could not be found, but invertase activities were significantly lower in embryos from coated seeds in these two species. In addition to genetic differences between the species, differences in the timing of imbibition and progressing germination may also have interfered with the measurements during the first 30h of germination.

4.1 Introduction

Seed coating with hydro-absorbers as a technology to overcome water related problems in drought-prone agricultural systems generally results in low or reduced germination rates (Berdahl and Barker 1980; Schneider and Renault 1997; Willenborg *et al*. 2004; Gorim and Asch 2012). Earlier studies attributed the low germination rates to the effects of the coating on water imbibition and available oxygen in the seed (Baxter and Waters 1986; Klein and Sachs 1992). In contrast, in those seeds germinating, hydro-absorber coated barley, rye and wheat seeds with coat shares greater than 75 % of the average seed have been shown to promote better seedling growth compared to those seedlings growing from uncoated seeds (Gorim and Asch 2012) by increasing efficiency of grain reserve mobilization and a switch from invertase-based to sucrose synthase-based embryonic sucrose breakdown (Gorim and Asch 2014). We have argued that this apparent switch in activity of sucrose metabolic enzymes could be brought upon through a reduced oxygen supply to the embryo due to a higher saturation of the seed with water supplied from the coat (Gorim and Asch 2014). In general, germination progresses in 3 phases that are delineated by water uptake characteristics. Germination begins with rapid imbibition of water in phase I followed by a plateau phase of seed moisture content in phase II during which the enzymatic and energetic basis for reserve mobilization is laid (Howell *et al*. 2009; Nonogaki *et al*. 2010; Weitbrecht *et al*. 2011). Phase III begins with the radicle emergence and is characterized by a rapid increase in seedling water content and massive reserve mobilization from storage tissues. In an earlier study (Gorim and Asch 2014) we reported on early seedling growth and sucrose and glucose mobilization during phase III of germination. When growing from coated seeds, barley, rye, and wheat differed strongly in mobilization efficiency and thus, the respiratory losses of reserves mobilized during germination as compared to seedlings growing from uncoated seed. This indicated a strong effect of the coating on the enzymatic break down of starch and sucrose during germination suggesting a major influence on the seed metabolism during phase II of germination (the first 48 hours of seed germination) when major enzymes involved in carbohydrate and protein metabolism are activated (Weitbrecht *et al*. 2011). Carbohydrate metabolism has been shown to be active one hour after imbibition based on metabolite levels suggesting an immediate increase in the activity of glycolysis and the TCA cycle that facilitates early energy- demanding processes (Howell *et al*. 2009; Sreenivasulu *et al*. 2008). Transcripts and mRNAs among others, encoding starch breakdown and several sucrose synthesizing and cleaving enzymes already present in dry seeds are activated during phase I and II of germination (Sreenivasulu *et al*. 2008), with the level of activation (up- or down regulation) depending on environmental conditions and the physiological state of the seed (Nonogaki *et al*. 2010). The degradation of sucrose is a major pathway fueling glycolysis (Koch 2004). Sucrose can be cleaved by either invertase (into glucose and fructose) or by sucrose synthase into fructose and uridine diphosphoglucose, depending on tissue oxygen availability (Perata *et al*. 1997; Koch 2004). The recent development of oxygen-sensitive micro-sensors allows measuring oxygen profiles in living tissues. So far, research focused mainly on developing and maturing seeds (Rolletschek *et al*. 2005, 2009) and little is known about the oxygen distribution in tissues of germinating cereal seeds.

For the current study, we hypothesize that coating strongly affects water uptake to the seed, this in turn strongly influences the oxygen availability in tissues critical for successful mobilization of endosperm reserves which in turn has an effect on the activities of the sucrose cleaving enzymes providing the developing embryo with the sugar required for growth. The focus of our study is therefore three pronged: (1) we investigated imbibition during the first 30 hours in seeds of wheat, rye and barley coated with the hydro-absorber, Stockosorb® compared to that of the uncoated seed employing the widely and successfully used (e.g. Maharaj and Sankat 2000; Turhan *et al*. 2002) imbibition model developed by Peleg (1988). (2) We determined the oxygen concentration in the various tissues of the germinating seed with and without coating. (3) We investigated sucrose and glucose abundance in early embryonic tissue with the aim to determine which enzymatic activity may govern sucrose metabolisms in either coated or uncoated seeds.

4.2 Materials and Methods

4.2.1 Plant material and treatments

Seeds of spring barley (*Hordeum vulgare* L., cv. Maltasia), winter rye (*Secale cereal* L., cv. Jobaro), and spring wheat (*Triticum aestivum* L., cv. Thasas) were obtained from Freudenberger Feldsaaten GmbH and used in all experiments. Seeds of all 3 cereal species were either uncoated or coated with a seed coat developed by Freudenberger Feldsaaten GmbH containing a specific amount of hydro-absorber (Stockosorb®). The term "coated" refers to grains with a coat share greater than 75 %. The term "uncoated" refers to the original seed.

4.2.2 Determination of grain initial moisture content

The initial moisture content of the grain was determined following the standard procedure laid down by ISTA (2010). For each cereal species randomly selected 25 coated grains, uncoated grains, and kernels from coat (coated grains of which the coat was removed) respectively were cut longitudinally using a sharp blade into halves and each half was then sliced four times. The sliced grains were immediately placed into 2 ml pre-weighed test tubes and weighed again. The samples were then transferred to an oven and dried at 103°C for 17 hours ±15 minutes and later cooled in a desiccator for 1 hour ± 15 minutes and weighed. The mass of the individual seeds was calculated by subtracting the mass of the empty test tubes from those of the test tube containing seeds. The amount of water lost was the difference between the weight in the pre-oven test tubes and post-oven test tubes. Therefore, the initial moisture content (MC_i) was the moisture difference divided by the seed mass expressed in percentage.

4.2.3 Estimating grain moisture content over time

25 grains were randomly selected for all cereals from all treatments and individually weighed on an electronic balance. Coated as well as uncoated seeds of each cereal were placed on moist filter paper (Ecolab-Bogen-Filterpapier, Neolab® Heidelberg,

Germany) in labeled plates 19.5 cm by 19.5 cm and plates were immediately transferred to the growth chambers (Percival Intellus® Ultra Controller) maintained at 25°C. Individual whole grains (WG) as well as the uncoated seeds were directly weighed every 3hours for the first 12 hours and subsequently every 6 hours until the 30th hour. After every weighing time, the 25 coated grains were quickly washed, blotted on filter paper to remove excess water and weighed to get a kernel from coat (SFG) weight. The difference between an individual grain weight at a given time t and its original weight at time t_0 divided by its weight at time t expressed as a percentage gives the MC at that point in time. The MC for the SFG was estimated based on the assumption that their initial weight was the same as that of an average seed.

4.2.4 Estimating grain imbibition rate and capacity

Knowing the initial MC and MC enabled the estimation of the imbibition rate and imbibition capacity from the model postulated by Peleg (1988) based on a two-parameter sorption equation, thus:

$$\mathrm{M_t} = M_0 \pm \frac{t}{K_1 + K_2 t} \qquad (1)$$

Where M_t is the MC of grains at time t in percentage, M_0 is the initial MC (%), K_1 is the Peleg rate constant (min $\%^{-1}$), and K_2 is the Peleg capacity constant ($\%^{-1}$). High values of K_1 or K_2 imply low imbibition rate (IR) and low capacity, respectively, and the inverse is true for low values of K_1 and K_2. Imbibition is an absorptive process and so, '±' in Eq. 1 becomes '+'. The imbibition or absorption rate (R) was obtained from the first derivative of equation Eq.1

$$R = \frac{dM}{dt} = \pm \frac{K_1}{(K_1 + K_2)^2} \qquad (2)$$

The Peleg rate constant K_1 is the inverse of the initial imbibition rate (IR) of grains (R_0) at time t_0. The Peleg imbibition capacity constant K_2 relates to the maximum or minimum attainable grain MC. As t →∞, Equation (1) gives the relation between grain equilibrium moisture content (M_e) and K_2.

$$M|t_\infty = M_e = M_0 \pm \frac{1}{K_2} \quad (3)$$

In order to obtain K_1 and K_2, Eq. 1 was linearized resulting in the following equation:

$$\frac{t}{M_t - M_0} = K_1 + K_2 t \quad (4)$$

A plot of t/M_t-M_0 versus time therefore gave a straight line, with K_1 being the y-intercept and K_2 the slope and from these constants, the equilibrium MC (M_e) and initial IR (R_0) were calculated.

The water absorption capacity and IR between coated and uncoated treatments were estimated by comparing the slopes of the straight lines produced when Eq. 4 was plotted. The imbibition rate was also expressed in terms of the amount of water imbibed by the grain per hour.

4.2.5 Measurement of oxygen in seed from grain and hydro-absorber coating

The oxygen concentrations across a section of seeds coated with hydro-absorber were determined for barley, rye and wheat using a less than 50 µm tip glass fibre needle-type oxygen micro-sensor (micro-sensor, Presens, Regensburg, Germany) connected to a fibre optic oxygen meter (Microx TX3, Presens) with the sensor mounted on a micro-manipulator as explained by Rolletschek *et al* (2002). Before measurements began, the oxygen sensor together with the temperature sensor was calibrated in oxygen free sodium sulfite (Na_2SO_3) dissolved in 100 mL distilled water and later in normal water to ensure temperature compensation during measurements. Hydro-absorber coated grains with coat shares > 75% were randomly selected and grains placed intermittently every 30 minutes interval at marked positions on moist filter paper and immediately transferred to the growth chambers at 25°C. At 18 hours and then every 6 hours, grains were removed in the sequence at which they were placed in the plates, the hydro-absorber coating was quickly washed off and excess water had been blotted from them. Their oxygen profiles were measured by mounting the kerned on a micro-manipulator stage and driving the oxygen needle tip at 50, 100 and then 200 µm distances into the embryo from the top and through the scutellum with the aid of the micro-manipulator; the probe extended, readings allowed to stabilize and recorded after which the needle was driven to a new position and the process repeated. (Fig. 1) The path of the needle was verified and confirmed by dissecting each seed at the end of each measurement and viewing under a microscope. In order to measure the amount of oxygen in the coating, the same process was repeated but the hydro-absorber coating was not washed off and the whole grain was mounted. In order to protect the needled tip the whole capsule was moved into the coating and the needle tip extended in a controlled manner only at the sites where measurements we demanded. The thickness of coating, testa and endosperm was determined by observing at least 5 representative seeds at 10 fold magnification under a stereo microscope (Leitz BioMeD) calibrated with a stage micrometer scale. Data is presented as means of at least 6 grains and oxygen was recorded as percentage saturation.

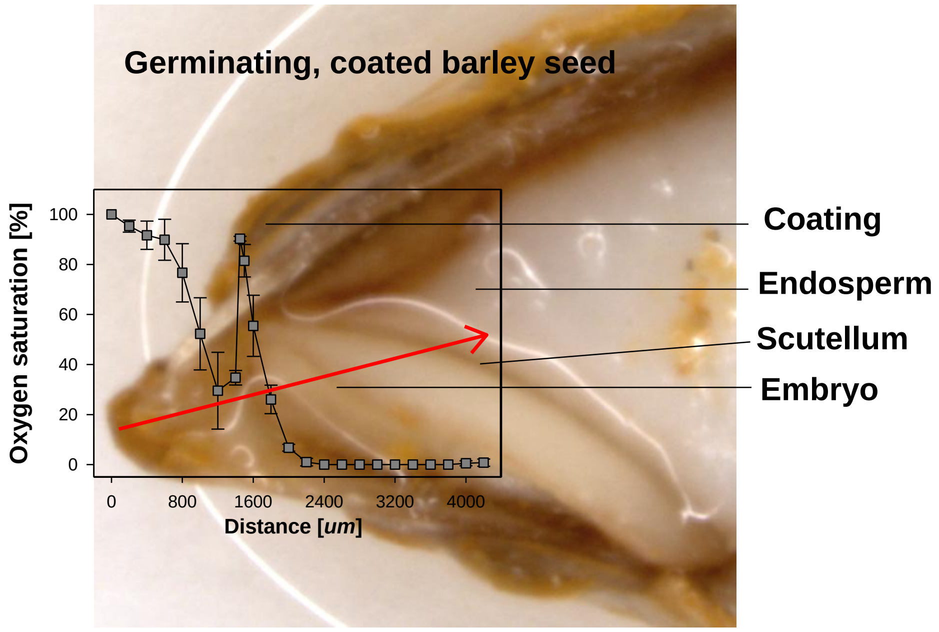


Fig. 1 Exemplary illustration of an oxygen profile through the coating, the seed coat and the embryo into the endosperm. The path of the micro-probe is indicated by the arrow.

4.2.6 Preparation of plant materials for sugars analysis

Barley, rye and wheat seeds, both coated and uncoated were place on moist filter paper in plates as previously reported in Gorim and Asch, (2012). Every 6 hours from the 12th hour, the hydro-absorber coating was washed off the coated seed and in both coated and uncoated seeds, embryonic material was quickly excised with a stainless steel razor blade on ice, and freeze dried in 2 mL Eppendorf vials.

4.2.6.1 Extraction and quantification of sugars

Ten milligrams of freeze dried embryonic material per replicate were ground in 'FastPrep Lysing matrix A' tubes at 6.0 MS for 40 seconds in the FastPrep® - 24 homogenizer. 500 µL 80% ethanol was added, the tubes vortex and heated at 60 °C for 30 minutes. Each tube was centrifuged for 10 minutes at 13,000g and the supernatant transferred to another Eppendorf-vial. The process, from addition of ethanol to centrifugation was repeated twice. The ethanol extract was evaporated to dryness in a vacuum centrifuge (Eppendorf Concentrator 5301) at 45 °C for 2.5 h and re-dissolved in 900 µL water by shaking over night. Sugars were assayed as described by Andersen *et al*. (2002). For glucose, 100 µL were assayed in a total volume of 200 µL water. The dye, glucose oxidase/peroxidase/ABTS-solution (400 µL) was added and samples incubated in a water bath at 37°C for 30 minutes.

For sucrose plus glucose, 50 µL were assayed in a total volume of 300 µL in NaAc-buffer containing 50 mM Na-acetate and 15 mM magnesium chloride (pH 4.6). Invertase solution, 2 µL and the dye were added. Samples were read at 418 nm in a spectrophotometer (Beckmann, Type UV-Du640, USA).

4.2.6.2 Calculation of the amount of sugars

From the linear standard curve, the amount of sugars in each sample measured per milligram dry matter or milligram reserves remobilized was calculated. For example: The amount of glucose per milligram sample (mg/mg) in an unknown sample

$$x = \frac{[0.300\mathrm{ml}].\ \left\lfloor \mathrm{Y1}\left(\frac{\mathrm{mg}}{\mathrm{ml}}\right)\right\rfloor [900\mathrm{ul}]}{[50uL].\ [Zmg]}$$

Where:

x is the amount of sugar to be calculated

Y1 (mg/ml) is the concentration of glucose obtained from the standard curve

0.300ml. [Y1 (mg/ml)] is the amount of glucose in 300 µL assayed (mg)

[0.300ml. [Y1 (mg/ml)].[(0.900/50 µL)] is the amount of glucose in the total (900µL) samples

Z mg can either be weight lost (∂) from grain or increased in dry matter (∂_1)

4.2.7 Quantification of enzymatic activity

Crude enzyme extracts from approximately 10 mg of freeze dried and frozen material replicated 3 times were ground in 'FastPrep® Lysing matrix A' tubes at speeds of 6.0 ms^{-1} for 40 s in the FastPrep-24 machine. 500 µL of extraction buffer consisting of 50 mM HEPES-NaOH, 1 mM EDTA, and 2.5 mM dithiothreitol, (pH 7.0) was added to the samples that were vortex. The samples were then centrifuged for 10 min at 20,000g to pellet insoluble material. The soluble protein extract was removed and insoluble proteins extracted with buffer containing 1 M NaCl, 50 mM HEPES-NaOH, 1 mM EDTA, and 2.5 mM dithiothreitol, pH 7.0. Soluble protein extract (400 µL) was dialyzed against extraction buffer for 20 h at 0°C on a 14.000 MWCO dialysis membrane (Visking Karlsruhe, Germany) to remove endogenous soluble carbohydrates. The concentration of total protein was measured in the extract as described by Bradford (1976) using a bovine serum albumin (BSA) standard.

Activities of soluble acid invertase and insoluble acid invertase were measured as described by Tsai *et al*. (1970) with minor modifications. Invertase extracts (20 µL) were assayed in a total volume of 300 µL, with an assay buffer containing 50 mM sodium acetate, 15 mM magnesium chloride, and 100 mM sucrose (pH 5.0). Assays were incubated for 1 h at 30 °C, with blank terminated immediately after addition of protein extracts. All reactions were terminated by boiling and 400 µL glucose oxidase/peroxidase/ABTS-solution added to all tubes that were incubated at 37 °C in a water bath for 30 minutes. Samples were read in a spectrophotometer (Beckman, Type UV-Du640, USA) at a wavelength of 418 nm. Three samples were assayed for each grain category with duplicate quantification of each.

4.3 Results

4.3.1 Effects of coating on imbibition

In order to germinate, seeds imbibe water. The entry of water into seeds increases their moisture linearly which later plateaus at equilibrium moisture content (Peleg 1988; Turhan *et al*. 2002). This pattern was also observed in the current study for barley, rye and wheat seeds. Barley and wheat seeds from within the coat had higher moisture contents compared to their uncoated counterparts (Fig. $2_{a\text{-}c}$). This was reflected by the high imbibition rate constant (K_1) for seeds within the coat while their imbibition capacity constant, K_2 was consistently lower (Table 1). The initial absorption rate was higher in the coated seeds (entire grain) in all 3 cereal species as compared to the uncoated seeds but the seeds within the coats had the lowest initial absorption rates (Table 1). The initial moisture content of the seeds within the coat was increased by coating in rye and wheat but not in barley. In comparison to the uncoated seeds, the equilibrium moisture content was increased in the seeds from within the coats in all 3 cereals with the largest increase (15.5%) in wheat and the smallest (4.5%) in barley (Table 1).

4.3.2 Oxygen profiles in coated and uncoated seed

Oxygen saturation levels were measured across coated and uncoated seeds of all three cereals through the embryonic tissue into the endosperm (Fig. 1) in regular intervals during the first 48 hours after soaking. The sensor was first inserted through the coating material until it reached the testa. Then, in order to avoid contaminations, the seed coat was washed of and the sensor was newly placed and inserted into the seed. Thus, the apparent increase of oxygen saturation between the coat and the seed in measurements from coated seeds is an artifact owed to the measuring technique (Fig. 3). In general, oxygen saturation decreased rapidly in the coat of coated seeds by 80-90% of atmospheric oxygen levels and remained at levels of 1-

5% in barley and rye embryos in seeds from within the coat and 8-10% in wheat embryos from seeds from within the coat (Fig. 3).

Table 1

Imbibition characteristics during the first 30 hours after soaking of coated and uncoated barley, rye, and wheat seeds. Coated refers to the entire grain including seed and coat, SFG refers to the seed within the coat; Uncoated refers to the original seed without coating. K_1 is the Peleg rate, K_2 the capacity constant, IC is the Imbibition capacity and M_e is the equilibrium or saturation moisture content. [Small letters refer to mean comparisons between SFG and the uncoated seed for each parameter analyzed at alpha equals 5% meanwhile capital letters denote differences between cereals for the same treatment for any given parameter at alpha equals 5%].

Cereals	Treatments	$K_1 \times 10^{-2}$ (hr $\%^{-1}$)	$K_2 \times 10^{-2}$ ($\%^{-1}$)	IC (%)	M_e (%)
Barley	SFG	5.0 (±0.8)^{aB}	2.5 (±0.1)^{aB}	40.4 (±0.8)^{aB}	51.1 (±0.8)^{aB}
	Uncoated	5.9 (±1.2)^{aB}	2.8 (±0.1)^{aB}	35.8 (±1.7)^{aA}	47.5 (±1.7)^{aA}
Rye	SFG	8.9 (±0.9)^{aA}	2.1(±0.1)^{bC}	47.1 (±2.1)^{aA}	58.3 (±2.1)^{aA}
	Uncoated	5.4 (±0.3)^{bB}	2.6 (±0.2)^{aB}	37.9 (±0.3)^{bA}	48.1 (±0.3)^{bA}
Wheat	SFG	2.5 (±0.6)^{bB}	2.8 (±0.1)^{bA}	36.2 (±1.0)^{aB}	47.0 (±1.0)^{aB}
	Uncoated	11.8(±2.1)^{aA}	4.1 (±0.2)^{aA}	24.3 (±0.9)^{bB}	33.9 (±0.9)^{bB}

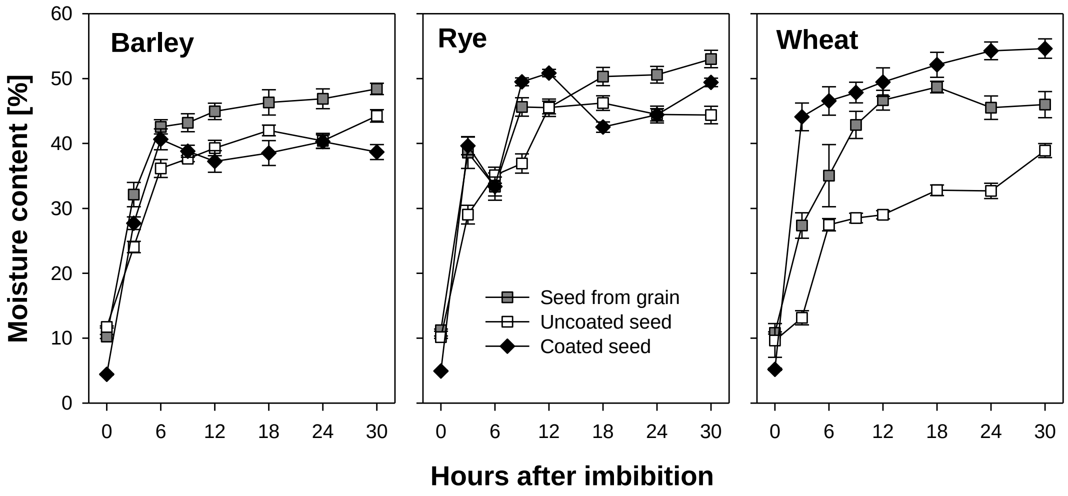


Fig. 2 Moisture content of seeds within the coat and uncoated seed during the first 30h after soaking in barley, rye and wheat. [Error bars represent standard error of means].

The three cereal species differed substantially in the oxygen saturation levels in the embryo in the uncoated seeds during the first 30h of germination. In barley average oxygen saturation in the embryo varied between 50 and 80% of atmospheric levels, in rye mean oxygen saturation was between 70 and 80% and in wheat oxygen saturation levels decreased with increasing distance into the embryo to levels of 20 – 30% with mean values at about 40%. In barley and rye endosperm oxygen saturation in coated and uncoated grains was between 1 and 10% whereas in wheat endosperm oxygen saturation in uncoated seeds was at about 20% and in coated seeds between 1 and 5%. After 48h the radicle had already broken through and coating at the position of sensor entry was no longer present. Still the embryonic oxygen saturation levels were strongly reduced to levels of 1-5% in coated seeds as compared to uncoated seeds in all three species. Theoretically, sucrose in either endosperm or scutellum is synthesized from starch breakdown and should thus be linearly related with endosperm mobilization, whereas glucose found in the embryo should be a product of sucrose cleavage in the embryo. If sucrose is cleaved into anything else but glucose and fructose for example UDP glucose, the analyses used in this study would not detect it as glucose. Figure 4 shows the relationship between mobilization of endosperm reserves and sucrose found in the respective embryos and the relationship between this sucrose and glucose found in the same tissues during the first 30 hours of germination. Sucrose in the embryo was linearly related to the amount of endosperm reserves mobilized in all three species and amounts were similar with minor differences between species (Fig 4 a-c). For glucose as related to sucrose, the situation was different. Since the method of sugar analyses used here only detects glucose but not UDP-glucose the relationship between glucose and sucrose mobilized found in barley for the coated and uncoated seeds was according to expectations. A strong linear relationship was found in the embryos from uncoated seed indicating an active invertase-based cleavage pathway for sucrose and less than a third of glucose was found in the embryos from coated seeds indicating an inhibition of the invertase-based pathway (Fig. 4d). For the embryos from coated seeds in rye and wheat a similar pattern was found (Fig. 4e-f), although in rye and wheat embryos from coated seeds smaller amounts of sucrose mobilized endosperm reserves were found when compared with the uncoated seed (Fig. 4b-c). However, in these species very little glucose was found in embryos from uncoated seeds, despite the fact that sucrose was amply available (Fig. 4e-f).

4.3.3 Effects of coating on soluble and insoluble invertase activity

The activity of soluble and insoluble invertase did not differ significantly in embryonic tissues of both coated and uncoated seeds during the first 48h (data not shown). However, when calculated as mean activity over 48 hours, the total invertase activity was higher in barley embryos from coated seeds than in embryos from uncoated seeds, but significantly lower in embryos from coated seeds in rye and wheat seeds (Fig. 5).

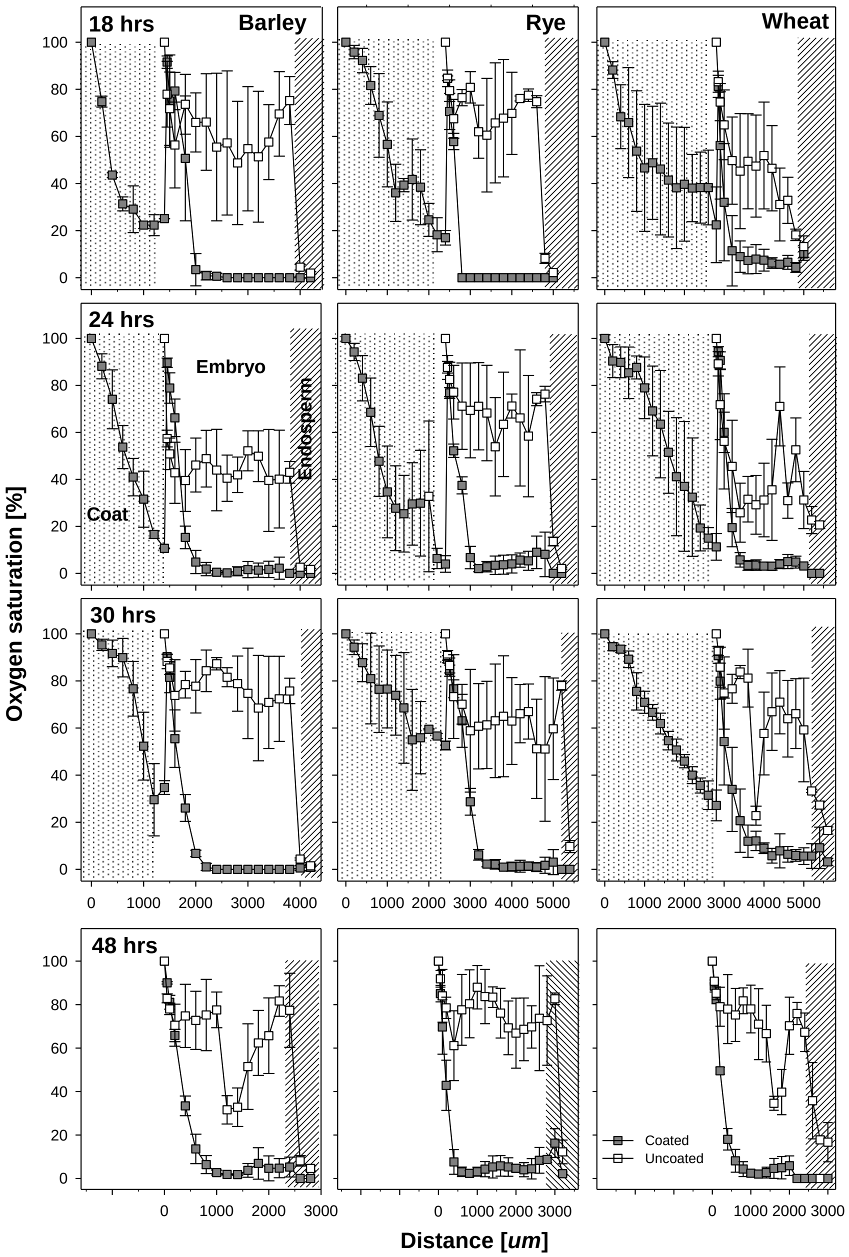


Fig. 3 Oxygen profiles taken as indicated in Figure 1 at 18, 24, 30, and 48 hours after soaking from coated and uncoated seeds of barley, rye, and wheat. The apparent increase of oxygen concentration after the coating material in the profiles from the coated seeds is an artifact due to the necessity to remove the coat before entering the micro-probe into the embryo. Coats where not present anymore at 48h after soaking [Error bars represent standard error of means].

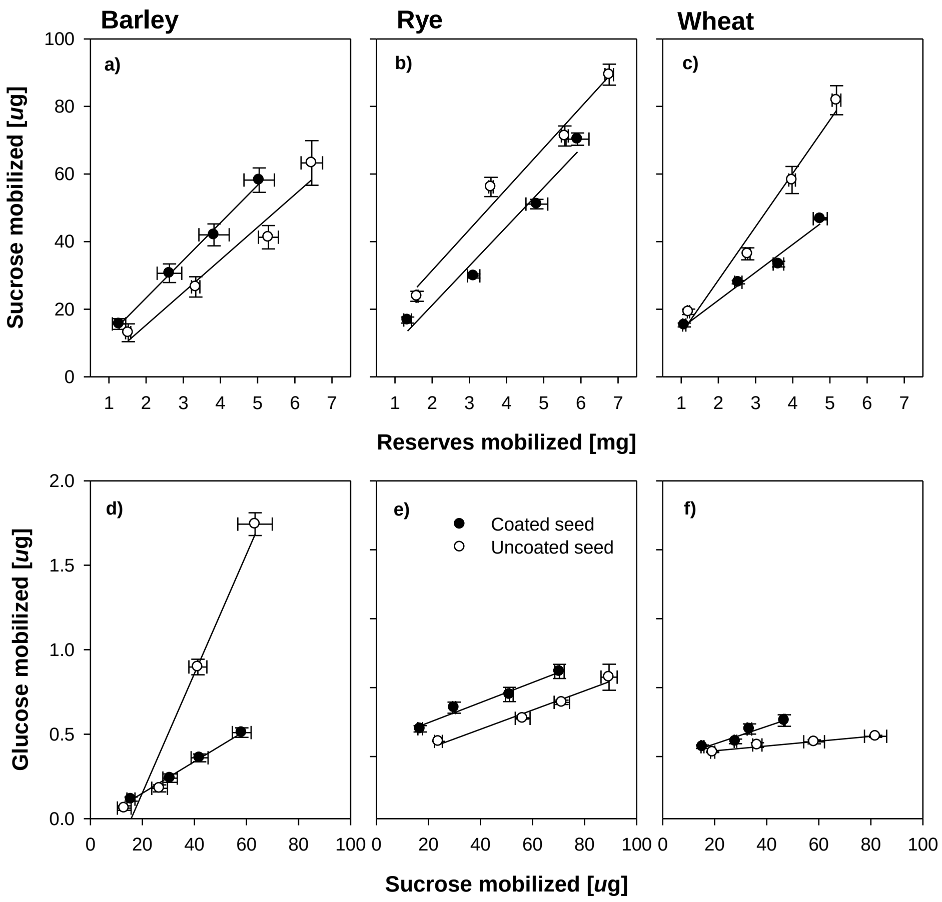


Fig. 4 Relationships between the amount of reserves mobilized from the grain and the amount of sucrose found in the embryo (a-c) and the relationship between the amount of sucrose and glucose found in the embryonic tissues (d-f) in the seed from within the coat and uncoated seeds of barley, rye, and wheat. [Error bars represent standard error of means].

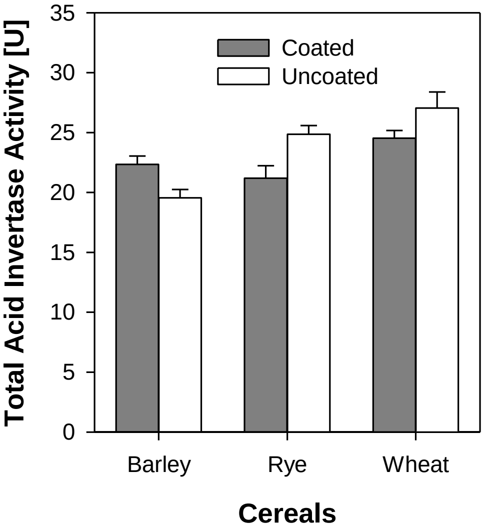


Fig. 5 Mean total acid invertase activity during the first 48h after soaking in embryonic tissue from seeds from within the coat and uncoated seeds of barley, rye and wheat [Error bars represent standard error of means].

4.4 Discussion

4.4.1 Effects of coating on water imbibition in cereals

We studied water uptake of both coated and uncoated seeds for 30 hours after imbibition. Imbibition begins during phase I of seed germination and continues through phase II (Howell *et al*. 2009). Initially, there is a rapid uptake of water mainly through the micropyle of the dry seed (phase I) until all of the matrices and cell contents are fully hydrated (Leopold 1983; Nonogaki *et al*. 2010; Weitbrecht *et al*. 2011). This behavior was observed in both uncoated seeds and the seeds from within the coats of barley, rye and wheat during imbibition however, the seeds from within the coat showed a much steeper initial uptake especially in wheat (Fig. 2). This phase lasted for about 3 and 6 hours in seeds from within the coat and uncoated barley and rye seeds respectively, whereas duration of phase I was 6 and 12 hours in wheat for uncoated seeds and seeds from within the coat respectively.

This delay in the entering into phase II in wheat in the coated seeds may play a role in the delayed germination that was subsequently observed (Gorim and Asch 2012). Although the moisture content of seeds from within the coat in these cereals was higher compared to that in the uncoated seeds, the imbibition rate was lower and the maximum imbibition capacity at any point was also lower in seeds from within the coat (Fig. 2; Table 1). The reason for this could be that the water absorbed through the coating dotted with hydro-absorber was channeled slowly to the kernel within resulting in fewer membranes being damaged in the seeds enveloped by this coat compared to those in uncoated seeds since it is known that rehydration imposes considerable stress upon cells components leading to the leakage of solutes indicative of temporal membrane damage (Nonogaki *et al*. 2010; Weitbrecht *et al*. 2011).

Seed moisture content dynamics plotted to determine the imbibition rate and capacity (data not shown) showed similar patterns in both uncoated seeds and seeds from within coats with initial sharp increases in the amount of water absorbed which later levels off at equilibrium with very high coefficient of determination (0.971-0.999) and this was in agreement with similar observations reported for other seeds (Turhan *et al*. 2002; Meyer *et al*. 2007). However, the initial imbibition rate was reduced seeds for within the coat in barley and rye (Table 1) resulting in less water absorbed into these seeds as imbibition progressed which resulted in very high equilibrium moisture contents. The difference in equilibrium moisture content between the seeds from within the coat and the uncoated seeds was 4.5%, 9.5% and 15.5% for barley, rye and wheat, respectively. The high equilibrium moisture content observed in rye and wheat could have led to over-saturation in the kernel within the hydro-absorber coating resulting in low oxygen conditions, interruption of most metabolic processes; probably leading to tissue damage (Leopold 1983; Nonogaki *et al*. 2010) and ultimately resulting in the poor germination rates reported in hydro-absorber coated wheat and, to a lesser extent, coated rye (Gorim and Asch 2012).

4.4.2 Effect of coating on oxygen availability

Oxygen concentrations in embryos have been investigated in maturing seeds with the aid of oxygen micro-sensors and in some cases reported in relation to the enzymes, invertase and sucrose synthase (Rolletschek *et al*. 2002, 2005, 2009). However, we investigated for the first time *in situ* the oxygen supply to the embryo in germinating seeds with the aid of oxygen micro-sensors. We measured oxygen concentrations through the center of the embryo passing through the scutellum in coated and uncoated seeds as well as in the hydro-absorber coating surrounding barley, rye and wheat seeds at the site above the embryo. Measurements were done in the embryo because it is the site where products resulting from endosperm reserve mobilization are channeled to for seedling growth (Fincher 1989; Aoki *et al*. 2006). The presence of an imbibing hydro-absorber coating around the kernel resulted in strongly hypoxic conditions in the embryo. Relative to atmospheric conditions, oxygen concentration decreased in the hydro-absorber coating by 70 to 90%. Since the coating material was completely saturated with water, atmospheric oxygen was not able to diffuse through this barrier to reach the seed within the coat. Thus, the coating and the water contained within effectively blocked the oxygen supply to the seed. Oxygen in the embryos of coated seeds was greatly reduced by 90-99% relative to atmospheric concentrations across species and time and about 45-75%, 60-70% and 35-65% in barley, rye and wheat, respectively, compared to their uncoated counterparts (Fig. 3). However, oxygen measurements were carried out mostly in seeds; coated or uncoated that would have germinated. Lower oxygen concentrations in the embryo do not imply that oxygen was completely lacking but that its amount was much reduced and with mobilization actively progressing, the amount available was quickly consumed. This was evident from oxygen profiles for tissues surrounding the embryo center (data not shown) which suggest that oxygen supply to and around the embryo vary depending on the position with respect to the embryo center and this also varied as imbibition progressed in both coated and uncoated seeds. The micropyle has been shown to be the main gateway through which water enters seeds (Rathjen *et al*. 2009) and may also serve as supplier of oxygen at the same time since the endosperm of maturing cereal grains has been shown to be almost anoxic (Rolletschek *et al*. 2005) as well as the endosperm of germinating grains as shown here (Fig. 3).

4.4.3 Effects of coating on sugar metabolism and enzyme activity

Sucrose stored in the aleurone layer and also produced from oil catabolism, is believed to be actively secreted into the endosperm and taken up by the embryo as an early energy source before starch breakdown becomes the dominant source of carbohydrate supply (Chrispeels *et al*. 1973). The oxygen concentration in the embryo affects the pathway of sucrose cleavage with hypoxic conditions resulting in inhibition of acid invertases and activation of sucrose synthase (Perata *et al*. 1997; Zeng *et al*. 1999; Koch 2004) whose level has been reported to be elevated in both barley and wheat embryos only but not at other sites (Perata *et al*.1997). This could easily show the importance of the pathway switch since the reserves mobilized from the endosperm were proportional to the sucrose accumulation found in the embryo indicating no inhibition of the starch break down during germination resulting from low oxygen levels. However, the argument would hold only for both cereals (i.e. barley and wheat) if the glucose accumulated in the embryos of the uncoated seeds would be proportional to the sucrose accumulated in the embryo whereas no proportionality would be observed between glucose and sucrose accumulation in the embryonic tissues of seed from within the coat; Which was the case for barley, but not for wheat (Fig. 4). This may have resulted from different duration of the phasing during early germination, particularly imbibition and phase I (Nonogaki *et al*. 2010; Weitbrecht *et al*. 2011), as described above, so the early germination phases may have not been fully aligned in relation to time, and consequently, sucrose transport or glycolysis in the embryonic tissue may have been delayed.

4.4.4 Effect of hydro-absorber coating on enzyme activity

We determined the activities of soluble and insoluble invertase during the first 48h after imbibition of all three cereal species for coated and uncoated seeds. Both, activity as well as amounts present in the embryonic tissue did not vary systematically between species or coatings (data not shown). Mean total invertase activity did show significant differences between species and coatings (Fig. 5), however, it was not conclusive with regard to glucose and sucrose availability in the embryo. It is possible, that we were not able to capture the depression of invertase activity that may have happened in vivo under strongly hypoxic conditions in the embryo as the activity measurements were performed in vitro under laboratory conditions with oxygen present during the analyses. The mean values we found are in contrast to the report by Guglielminetti *et al*. (1995) who reported very low invertase activity in barley and rye under anoxic conditions. We suggest that based on our knowledge of oxygen supply in the embryo, invertase was present but its activity was depressed or alternatively, both invertase and sucrose synthase might have been active at different times during imbibition.

4.5 Conclusion

As postulated earlier (Gorim and Asch 2014), we investigated moisture uptake to the uncoated seed and the seed within the coat as well as the oxygen profiles in the embryos of the respective seeds. We found that seeds within coatings absorbed significantly more moisture than uncoated seeds. Using a micro probe technology, we could for the first time provide evidence that coating result in near anoxic oxygen concentrations in the developing embryonic tissues in all three species. In barley, clearly sucrose was not cleaved via the invertase pathway anymore, despite the fact that invertase activity levels in coated seeds were increased. In rye and wheat, clear evidence from the sugar availability in the embryo could not be found, but invertase activities were significantly lower in embryos from coated seeds in these two species. In addition to genetic differences between the species, differences in the timing of imbibition and progressing germination may also have interfered with the measurements during the first 30h of germination. The data on the enzymatic activities remained elusive since it was not possible to measure enzymatic activities or abundance *in situ*. We could show that the functionality of the invertases was not compromised by the low oxygen environment. However, if the activity is actually depressed within the living tissue remains the objective of further studies.

4.6 References

Anonymous, 2010: The International Seed testing Association (ISTA). 2010: International Rules for Seed Testing. Zürichstr.50 CH-8303 Bassersdorf, Switzerland

Andersen, N.M., Asch, F., Wu, Y., Jensen, R.C., Naested, H., Mogensen, O.V. and Koch, E.K. 2002: Soluble invertase expression is an early target of drought stress during the critical, abortion-sensitive phase of young ovary development in maize. Plant Physiology 130: 591-604.

Aoki, N., Scofield, G.N., Wang, X.D., Offler, C.E., Patrick, J.W. and Furbank, R.T. 2006: Pathways of sugar transport in germinating wheat seeds. Plant Physiology 141: 1255-1263.

Baxter, L. and Waters, L. 1986: Effect of a hydrophilic polymer seed coating on the imbibitions, respiration and germination of sweet corn at four matrix potentials. Journal of American Society of Horticutural Science 111: 517-520.

Berdahl, J.D. and Barker, R.E. 1980: Germination and emergence of Russian Wildrye seeds coated with Hydrophilic materials. Agronomy Journal 72: 1006-1008.

Bradford, M.M. 1976: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal of Biochemistry 72: 248-254.

Chrispeels, M.J., Tenner, A.J. and Johnson, K.D. 1973: Synthesis and release of sucrose by the aleurone layer of barley: Regulation by gibberelic acid. Planta 113: 35-46.

Fincher, G.B. 1989: Molecular and cellular biology associated with endosperm mobilization in germinating cereal grains. Annual Review of Plant Physiology and Plant Molecular Biology 40: 305-346.

Gorim, L. and Asch, F. 2014: Seed coating reduces respiration losses and affects sugar metabolism during germination in cereals. Functional Plant Biology, in press.

Gorim, L. and Asch, F. 2012: Effects of composition and share of seed coatings on the mobilization efficiency of cereal seeds during germination. Journal of Agronomy and Crop Science 198: 81-91.

Guglielminetti, L., Perata, P. and Alpi, A. 1995: Effect of anoxia on carbohydrate metabolism in rice seedlings. Plant Physiology 108: 735-741.

Howell, K.A., Narsai, R., Carroll, A., Ivanova, A., Lohse, M., Usadel, B., Millar, A.H. and Whelan, J. 2009: Mapping metabolic and transcript temporal switches during germination in rice highlights specific transcription factors and the role of RNA instability in the germination process. Plant Physiology 149: 961–980.

Klein, J.D. and Sachs, M. 1992: Measurement of water uptake and volatile production by coated wheat seeds and subsequent seedling growth. Seed Science Technology 20:299-305.

Koch, K. 2004: Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Current Opinion in Plant Biology 7: 235-246.

Leopold, A. C.1983: Volumetric components of seed imbibition. Plant Physiology 73: 677-680.

Maharaj, V. and Sankat, C.K. 2000: Rehydration characteristics and quality of dehydrated dasheen leaves. Canadian Agricultural Engineering 42: 81-85.

Meyer, C., Steudle, E. and Peterson, C.A. 2007: Patterns and kinetics of water uptake by soybean seeds. Journal of Experimental Botany 58: 717-732.

Nonogaki, H., Bassel, G.W. and Bewley, J.D. 2010: Germination-still a mystery. Plant Science 179: 574-581.

Peleg, M. 1988: An Empirical Model for the description of Moisture Sorption curves. Journal of Food Science 53: 1216-1217.

Perata, P., Pozueta-Romero, J., Akazawa, T. and Yamaguchi, J. 1992: Effect of anoxia on starch breakdown in rice and wheat seeds. Planta 188: 611-618.

Perata, P., Guglielminetti, L. and Alpi, A. 1997: Mobilization of Endosperm Reserves in Cereal Seeds under Anoxia. Annals of Botany 79: 49-56.

Rathjen, J.R., Strounina, E.V. and Mares, D.J. 2009: Water movements into dormant and non dormant wheat (*Triticum aestivum* L.) grains. Journal of Experimental Botany 60: 1619-1631.

Rolletschek, H., Borisjuk, L., Koschorreck, M., Wobus, U. and Weber, H. 2002: Legume embryo develop in a hypoxic enviroment. Journal of Experimental Botany 53: 1099-1107.

Rolletschek, H., Koch, K., Wobus, U. and Borisjuk, L. 2005: Positional cue for the starch/lipid balance in maize kernels and resource partitioning to the embryo. The Plant Journal. 42:69-83.

Rolletschek, H., Stangelmayer, A. and Borisjuk, L. 2009: Methodology and significance of micro-sensor-based oxygen mapping in plant seeds-an overview. Sensors 9: 3218-3227.

Schneider, A. and Renault, P. 1997: Effects of coating on seed imbibitions: I. Model Estimates of water Transport Coefficient. Crop Science 37: 1841-1849.

Sreenivasulu, N., Usadel, B.,Winter, A., Radchuk, V., Scholz, U., Stein, N., Weschke, W., Strickert, M., Close, T.J., Stitt, M., Graner, A. and Wobus, v. 2008: Barley grain maturation and germination: Metabolic pathway and regulatory network commonalities and differences highlighted by new MapMan/PageMan profiling tools. Plant Physiology 146: 1738-1758.

Tsai, C.Y., Salamini, F. and Nelson, O.E. 1970: Enzymes in carbohydrate metabolism in the developing endosperm of maize. Plant Physiology 46: 299-306.

Sigmaplot 10 for windows (SYSTAT software Inc. Chicago)

Turhan, M., Sayar, S. and Gunasekaran, S. 2002: Application of Peleg model to study water absorption in chickpea during soaking. Journal of food engineering 53: 153-159.

Zeng, Y., Wu, Y., Avigne, T.W. and Koch, E. K. 1999: Rapid repression of maize invertase by low oxygen. Invertase/Sucrose synthase balance, sugar signaling potential and seedling survival. Plant Physiology 121: 599-608.

5 Seed coating with hydro-absorbers as potential mitigation of early season drought in two sorghum varieties (*Sorghum bicolor* L. Moench var. Susu & Piper)

[The contents of this chapter is in preparation for submission in the journal: Field Crops Research]

5.1 Introduction

Sorghum is one of the most important staple food crops especially in the drier and more marginal areas of the semi-arid Tropics of Africa (FAO 1996; Ali *et al*. 2011). Sorghum is grown as a rain-fed crop sown after the first rains at the beginning of the rainy season. Climate change threatens sorghum production systems due to a shift of the onset of the rainy season to a later date (van de Giessen *et al*. 2010) accompanied by a larger variability in the time interval between the first and the second rain, resulting in a higher risk of crop failure during crop establishment (Volaire 2003; Marteau *et al*. 2011). Sorghum has been reported to be susceptible to variable soil moisture content during germination (Smith *et al*. 1988; Takele 2000) but to be highly resistant to drought stress at the seedling stage (Nour *et al*. 1978). Whereas sorghum drought tolerance traits and characteristics have been intensively studied for the later vegetative stages, flowering, and grain yield (e.g. Borrell *et al*. 2004; Patil 2007; Mutava *et al*. 2011; Sabadin *et al*. 2012; Abdulai *et al*. 2012), drought effects on sorghum during the critical period of seedling establishment have not been reported to date. In order to mitigate the potentially detrimental effects of variable rainfall during seedling establishment, it is important to improve understanding of seedling responses to dehydration between germination and crop establishment. One way to improve systems resilience to drought could be the identification of adapted genotypes while another way is to develop options to improve seedlings supply with water during the establishment phase. Coating seeds with a water absorbing substance offers a way to buffer the seed against insufficient moisture in the surrounding soil. Seed coating technology has been employed for different purposes with different substances for several decades. Coats may contain nutrients (Scott *et al*. 1987; Hassan *et al*. 1990), peroxides to provide oxygen, hormones to improve growth (Powell and Mathew, 1988) or hydro-absorbers to improve water supply (Kaufman 1991; Gorim and Asch 2012). Geohumus® and Stockosorb® are two commercially available hydro-absorbers that have the capacity to store a multiple of their weight in water. Sorghum seeds of two contrasting varieties were coated using the Mantelsaat technology of Freudenberger Feldsaaten GmbH including either of the hydro-absorbers. The aim of this study was to study the effects of those hydro-absorbers in the coating on early seedling growth of two sorghum varieties under different levels of drought stress. Takele (2000) proposed seedling shoot dry weight, root length, and seedling leaf area as screening tool for seedling drought tolerance in sorghum. In this study, we investigated the proposed parameters for the two varieties with coated and uncoated seeds at different levels of soil moisture deficit. The main hypotheses were (a) grains coated with the additional hydro-absorber performed better under drought stress as the coats can store moisture during the critical period of seedling establishment resulting in better seedling survival and improved growth after rewatering and (b) varieties will respond differently to both drought stress and hydro-absorber coatings.

5.2 Materials and methods

5.2.1 Plant materials and growth conditions

Seeds of two sorghum varieties; *Sorghum bicolor* L. Moench cv. Susu and *Sorghum bicolor* L. Moench cv. Piper were obtained from Freudenberger Feldsaaten GmbH and used in all experiments. Coatings for each variety comprised (i) seeds that were natural (uncoated), (ii) seeds coated with Stockosorb**®** and (iii) seeds coated with Geohumus**®,** both hydro-absorber containing coats were developed by Freudenberger Feldsaaten GmbH. The experiments were carried out in the greenhouse of the University of Hohenheim, Stuttgart, Germany from July to August of 2010 and 2011. The mean temperature and relative humidity ranged from

26.5±4.3°C to 28.2±5.4°C and 48.4±13.9% to 53.2±17.6% from July to August 2010 and 24.3±5.1°C to 27.9±6.5°C and 48.6±17.2% to 53.7±16.2% from July to August 2011.

5.2.2 The mass of sorghum seeds

The average mass of the uncoated seed was 22.5 (± 3.9) mg and 9.9 (± 1.1) mg for Susu and Piper, respectively. Seeds with Stockosorb included in the coat had an average mass of 65.9 (±13.5) mg and 35.4 (± 2.4) mg whereas when Geohumus was included in the coat, the coated seeds had an average mass of 71.6 (±23.0) mg and 37.1 (±11.6) mg for Susu and Piper, respectively.

5.2.3 Experimental setup

Pots (11x11x20cm) filled with medium coarse sand (2 kg) to which 60 mL Clark (1982) nutrient solution (diluted 1:5) were added were used in all experiments. Each pot contained seedlings (number per pot depending on the experiment) of each variety and coating type. For each combination, 3 replicates were established under fully watered conditions termed field capacity (FC = 20% moisture content) and at 2 moisture deficit levels: 50% FC (moderate drought) and 25% FC (severe drought) (Fig. 1). All pots were maintained at these moisture levels through difference weighing and appropriate addition of water loss twice daily.

5.2.4 Determination of early seedling root length and dry matter

Every other day from the fourth day after sowing (DAS), seedlings resulting from coated and uncoated seeds from 3 pots containing either Stockosorb, Geohumus, or uncoated seedlings were randomly chosen and their roots washed clean of soil. The root length of the individual seedlings from each pot was measured with a ruler, the seedling were separated into root and shoot and the samples oven-dried at 70 °C to constant weight. Samples were weighed using a fine balance and root/shoot ratio and total biomass determined.

5.2.5 Determination of plant height and leaf parameters

The experimental setup employed here constituted 8 seeds that were planted, 2 at 4 spots, 7 *x* 7 cm apart, at the center of each pot and later thinned to 4 plants per pot i.e. one at each spot. The plant height from ground level to the tallest leaf tip was determined at 10 DAS for both varieties. The leaf length (from base to tip) and widest width was measured daily for the second and third leaves for both varieties with the aid of a ruler from the 4 DAS onwards allowing determination of leaf elongation rate and leaf area. The leaf area of 5 random leaves from each treatment was determined using the leaf area meter (MK2, Delta-T, England) and their length (L) and width (W) measured with a ruler in other to estimate the multiplying factor (F) required to estimate total leaf area. The leaf area (y) was calculated from the formula: $y = L \times W \times F$. The relative leaf expansion rate (RLER) per day was calculated from the formula by Ober and Luterbacher (2002) thus: $\text{RLER} = (A_2 - A_1) / (t_2 - t_1)$ where A is the estimated leaf area and t is time. The leaf width measurements began when at least 80% of the leaf had unfolded. The lengths of the second, third and in some cases fourth leaves of seedlings depending on variety were measured. The leaf elongation rate (LER) was therefore the change in leaf length between on the length at any given day and that at 4 DAS divided by the time in between measurements in days.

5.2.6 Statistical analysis

Least significant differences were calculated with an analysis of variance at 5% alpha using the LSmeans and means statement in the general linear model (GLM) procedure of SAS. Graphs were drawn with the aid of Sigma Plot 10.

5.3 Results

5.3.1 Coating effects on seedling growth

The addition of hydro-absorber coating to seeds affected the growth of seedlings of either variety independent of soil moisture deficit. Under FC both coatings reduced the total biomass produced, LA and the root:shoot ratio in Piper compared to that of the uncoated seeds (Fig. 1). Stockosorb containing coats slightly enhanced root length in Piper, whereas coating with Geohumus resulted in significantly reduced root length when compared to the uncoated seeds (Fig. 1_{j-l}). Susu seedlings on the other hand responded differently to coating treatments. At field capacity, Stockosorb containing coats did not negatively influence biomass, LA, root:shoot ratio and root length which were decreased in seeds coated with Geohumus (Fig. 2). Both coatings significantly reduced seedling height at FC in Piper although similar seedling heights were observed under drought stress (Fig. 3). Seedlings growing from Susu seeds having Stockosorb in their coats were tall irrespective of the moisture content meanwhile those resulting from Geohumus coated seeds promoted growth under drought conditions and not when fully watered (Fig. 2).Under fully watered conditions, the second and third leaf elongation rate was reduced in seedlings growing from Geohumus coated Susu seeds meanwhile in seedlings growing from Piper coated seeds, the second leaf elongation rate was reduced by both coatings while the third leaf elongation rate was reduced by both coatings only at the 11 and 13 days after soaking (Table I). Expansion rate of the second and third leaves was higher in seedlings resulting from Geohumus coated Susu seeds meanwhile in Piper significant reduction in expansion rate were observed when seedlings were coated with Stockosorb (Table II).

5.3.2 Drought effects on seedling growth

Water availability strongly influenced early seedling growth in both varieties, however, responses varied among the varieties. More biomass was produced over time by Susu seedlings compared to those of Piper under drought conditions (Fig. $1_{a,b}$).

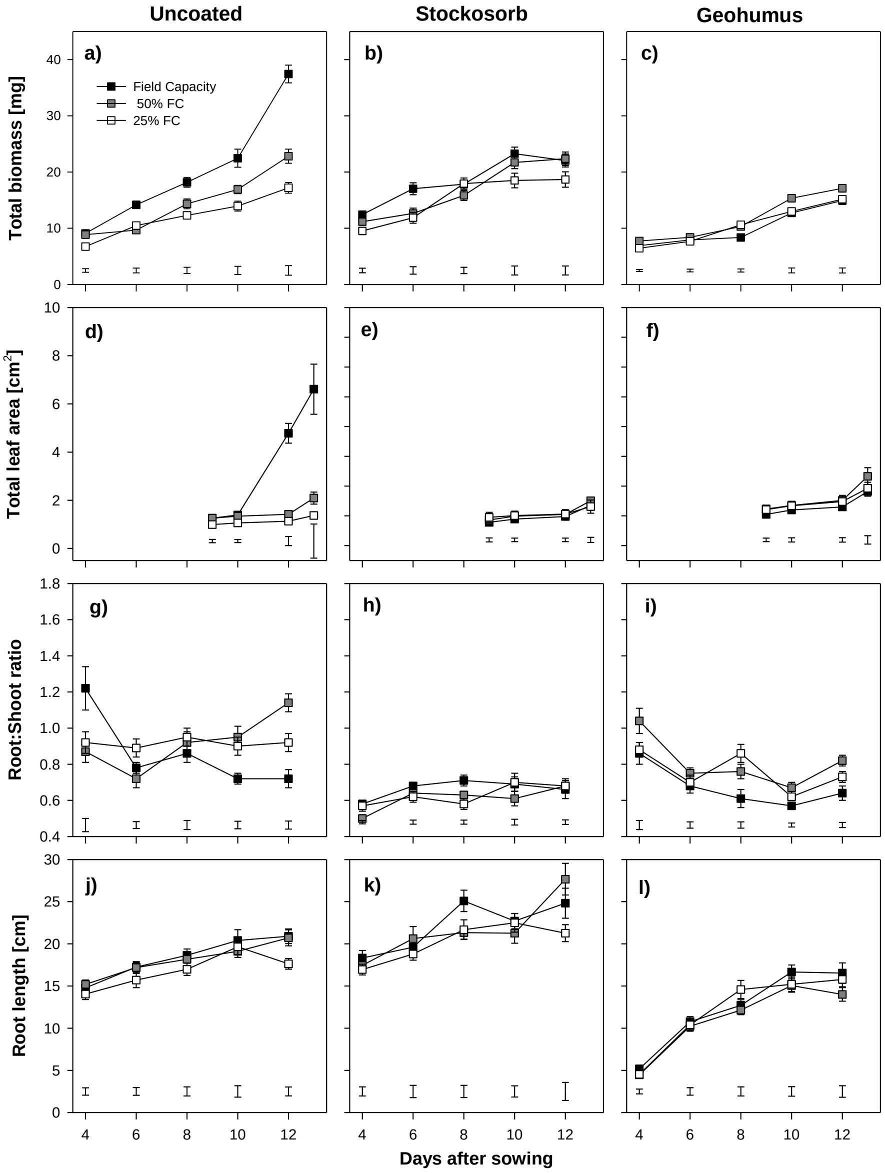


Fig. 1 The total plant biomass, leaf area, root:shoot ratio and root length in sorghum variety (Piper) seedlings from seeds coated with either Geohumus or Stockosorb as well as the uncoated seed and grown under 2 levels of drought and fully watered conditions. Bars on graphs represent the standard error of means and bars at the bottom of each graph represent the least significant difference at α = 5 %

Piper seedlings growing from the uncoated seed showed the expected pattern with fully watered seedlings producing the most biomass and those most stressed producing the least (Fig. 1a). In contrast, the highest amount of biomass over time in Susu was produced by seedlings growing at 50% FC (Fig. 2_a). However, total biomass was reduced by more than 60% in Piper and about 50% in Susu for seedlings growing from uncoated seeds at 25% FC (Fig. 1_a & 3_a). The total leaf area was reduced by 75% in seedlings growing from Piper coated seeds irrespective of the coating (Fig. $1_{h,i}$) meanwhile Susu coated seeds performed better irrespective of the coating (Fig. $2_{h,i}$). In seedlings growing from Piper coated seeds, the root:shoot ratio was reduced especially when Stockosorb was the coating used (Fig. 1_e) whereas the reverse behaviour was observed in seedling growing from Susu coated seeds (Fig. 2_f). In both varieties, root length was unaffected by water deficit in seedlings growing from uncoated seeds although seedlings growing from Susu coated seeds generally had significantly longer roots (Fig. 1j and 2j). Under drought, Stockosorb compared to Geohumus promoted root development in both varieties. Under drought, both coatings promoted plant height in seedlings growing from Susu coated seeds while those in Piper were unaffected (Fig. 3). Geohumus promoted leaf elongation rate irrespective of the level of drought in seedlings from Susu coated seeds while in Piper, seedlings from uncoated seeds had the highest second leaf elongation rate at 50% FC compared to those from both coatings although no differences were found between treatments for the third leaves at this drought level.

The third leaves of seedlings growing from Stockosorb coated seeds showed significantly low leaf elongation rate at 25% FC compared to the others (Table I). The Second leaf expansion rate was significantly higher in seedlings growing from Geohumus Susu coated seeds as well as in the third leaf at 13 days after soaking while at the other levels of drought, similar expansion were observed (Table II).

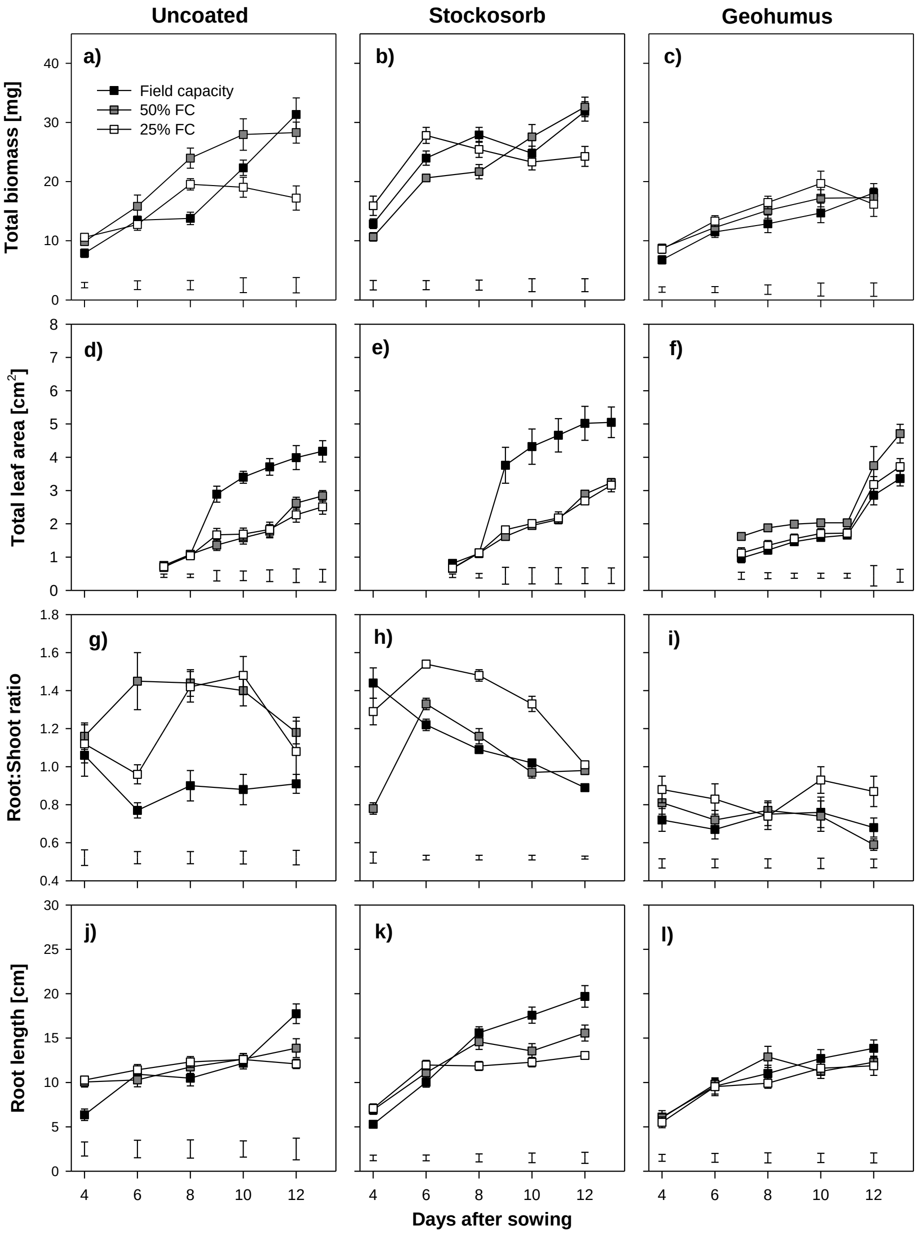


Fig. 2 The total plant biomass, leaf area, root:shoot ratio and root length in the sorghum variety (Susu) seedlings from seeds coated with either Geohumus or Stockosorb as well as the uncoated seed and grown under 2 levels of drought and fully watered conditions. Bars on graph represent the standard error of means and bars at the bottom of each graph represent the least significant difference at α = 5 %

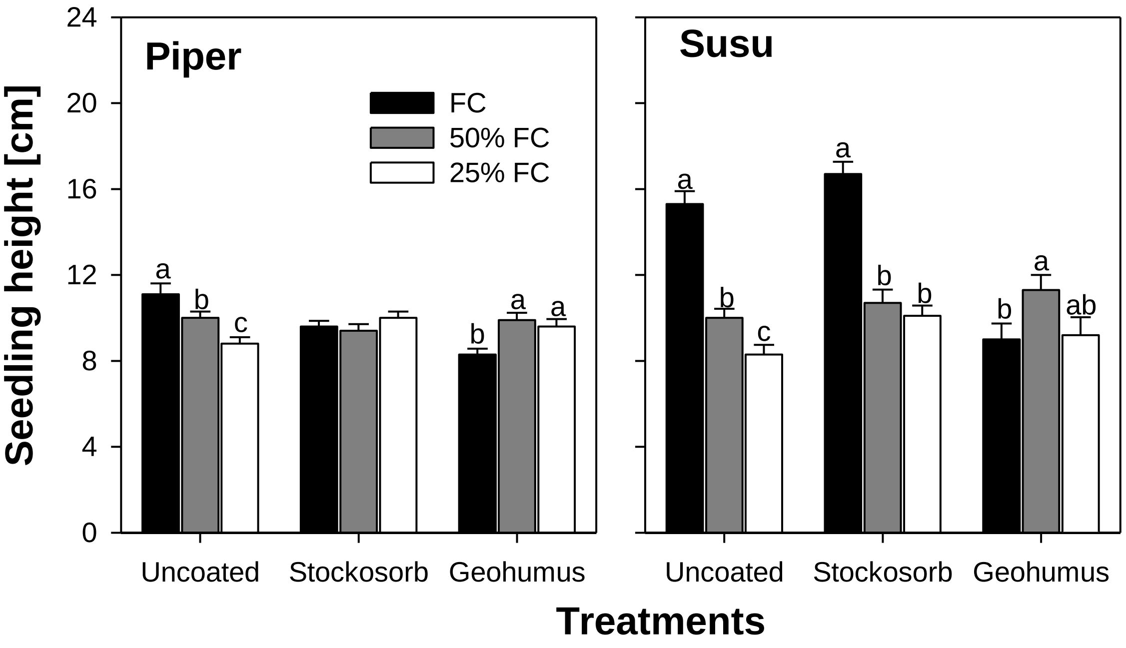


Fig. 3 Plant stem height in 2 sorghum varieties (Susu and Piper) seedlings growing from either uncoated seeds or from seeds coated with the hydro-absorber Geohumus or Stockosorb and seedlings grown under 2 drought level and fully watered conditions [Bar represent standard error of means and letters on bars significance at α = 5 %]. FC: Fully watered conditions.

Seedlings growing from Geohumus coated Piper seeds showed significantly high second leaf expansion rate at 25% FC at 8 days after soaking. Although the fully water seedling growing from the uncoated Piper seeds were the first to unfurl, leaf expansion rates were similar between treatments under drought (Table II).

5.4 Discussion

The uncoated Susu seed was significantly larger than Piper seed and this was true also when they were coated. This difference in seed mass may later have an effect since seed mass is known to affect the partitioning of resources especially during drought Khurana and Singh (2000).

Table I

Second and third leaf elongation rates (mm/day) of Susu and Piper seedlings at different moisture levels. Small and capital letters represents comparison in rates between moisture levels (rows) and comparisons between cereals at the same moisture level for a given day after sowing(DAS) respectively at alpha= 5%. Values in brackets are standard errors.

	Treatments								
	Uncoated			Stockosorb			Geohumus		
	Moisture levels								
DAS	FC	50% FC	25% FC	FC	50% FC	25% FC	FC	50% FC	25% FC
a) Second leaves									
6	6.1(±1.0)^{aA}	4.7(±0.6)^{aB}	4.6(±0.7)^{aA}	5.5(±0.8)^{aA}	6.3(±0.8)^{aB}	6.5(±1.0)^{aA}	7.2(±0.5)^{aA}	8.4(±0.9)^{aA}	7.4(±1.0)^{aA}
8	3.9(±0.6)^{aA}	3.1(±0.3)^{abB}	2.9(±0.4)^{bB}	3.8(±0.4)^{aB}	4.7(±0.4)^{aAB}	3.7(±0.5)^{aB}	5.2(±0.4)^{aB}	4.9(±0.4)^{aA}	6.2(±0.7)^{aA}
10	3.3(±0.5)^{aB}	2.9(±0.3)^{aB}	2.6(±0.3)^{aB}	3.3(±0.4)^{aB}	4.0(±0.3)^{aA}	3.5(±0.5)^{aB}	4.5(±0.4)^{abA}	4.0(±0.3)^{bA}	5.6(±0.7)^{aA}
b) Third leaves									
9	26.7(±2.2)^{aA}	11.3(±1.3)^{bA}	12.6(±1.0)^{bAB}	27.6(±1.8)^{aA}	14.3(±2.4)^{bA}	13.9(±1.0)^{bA}	12.0(±1.3)^{aB}	9.5(±0.7)^{aA}	10.0(±1.0)^{aB}
11	13.2(±0.8)^{aA}	5.3(±0.6)^{bB}	5.9(±0.6)^{bB}	14.1(±0.9)^{aA}	6.9(±0.9)^{abB}	7.1(±0.4)^{bAB}	9.2(±0.9)^{aB}	10.0(±0.5)^{aA}	8.8(±0.8)^{aA}
13	11.4(±0.8)^{aA}	6.8(±0.6)^{bB}	5.9(±0.6)^{bB}	11.3(±0.6)^{aA}	7.5(±0.7)^{abAB}	6.5(±0.3)^{bAB}	8.1(±0.6)^{abB}	8.6(±0.4)^{aA}	7.0(±0.5)^{bA}
c) Second Leaves									
6	15.3(±1.7)^{aA}	16.4(±1.1)^{aA}	13.9(±0.6)^{aA}	6.6(±0.9)^{aC}	8.7(±0.7)^{aB}	9.3(±1.6)^{aB}	10.3(±1.2)^{aB}	9.8(±1.0)^{aB}	9.3(±0.7)^{aB}
8	8.5(±0.3)^{aA}	8.2(±0.4)^{abA}	6.8(±0.3)^{bA}	5.9(±0.3)^{aB}	5.1(±0.4)^{bC}	5.8(±0.5)^{aA}	6.2(±0.3)^{aB}	6.6(±0.8)^{aB}	6.4(±0.4)^{aA}
10	8.0(±0.4)^{aA}	7.2(±0.4)^{aA}	6.1(±0.2)^{bA}	4.5(±0.2)^{aB}	3.7(±0.3)^{aB}	4.1(±0.3)^{aA}	4.6(±0.3)^{aB}	5.0(±0.7)^{aA}	4.8(±0.3)^{aA}
d) Third leaves									
9	16.8(±1.6)^{aA}	13.0(±1.4)^{abA}	7.9(±1.3)^{bB}	17.3(±1.6)^{aA}	12.5(±2.4)^{aA}	13.3(±1.9)^{aAB}	13.0(±1.7)^{aA}	15.8(±1.3)^{aA}	16.9(±2.2)^{aA}
11	17.8(±1.0)^{aA}	10.2(±0.9)^{bA}	9.7(±1.4)^{bA}	12.4(±0.9)^{aB}	10.4(±0.9)^{aA}	6.8(±0.6)^{bA}	10.3(±0.9)^{aB}	11.0(±0.8)^{aA}	9.8(±0.8)^{aA}
13	14.6(±0.6)^{aA}	8.7(±0.6)^{bA}	6.3(±0.6)^{cA}	9.8(±0.9)^{aB}	9.5(±0.7)^{abA}	6.9(±1.1)^{bA}	9.3(±1.9)^{aB}	9.5(±0.7)^{aA}	7.4(±0.5)^{aA}

5.4.1 Drought effect on seedlings growing from uncoated seeds

Growth of both the above and below ground parameters of the seedlings growing from uncoated seed of both varieties was suppressed by drought in our experiment. Both varieties showed little variation in root length at the 3 moisture levels investigated, with seedlings growing from Piper seeds at 25% FC shortest root length.

Table II

Second and third relative leaf expansion rate (mm^2/day) of Susu and Piper seedlings at different moisture levels. Small and capital letters represents comparison in rates between moisture levels (rows) and comparisons between cereals at the same moisture level for a given day after sowing(DAS) respectively at alpha= 5%. Values in brackets are standard errors.

	Treatments								
	Uncoated			Stockosorb			Geohumus		
	Moisture levels								
DAS	FC	50% FC	25% FC	FC	50% FC	25% FC	FC	50% FC	25% FC
a) Second leaves									
6	3.3(±0.4)^{aA}	3.9(±0.6)^{aA}	3.3(±0.3)^{aAB}	3.0(±0.8)^{aA}	2.7(±0.2)^{aAA}	4.7(±0.6)^{aA}	2.5(±0.4)^{aA}	2.7(±0.8)^{aA}	2.3(±0.4)^{aB}
8	0.6(±0.0)^{aB}	0.1(±0.1)^{aA}	0.1(±0.0)^{aB}	0.1(±0.0)^{bB}	0.5(±0.1)^{aA}	0.1(±0.0)^{bB}	1.2(±0.4)^{aA}	0.5(±0.2)^{aA}	1.0(±0.1)^{aA}
10	0.1(±0.1)^{bA}	0.0(±0.0)^{bA}	0.5(±0.2)^{bA}	0.4(±0.2)^{aA}	0.2(±0.0)^{aA}	0.2(±0.1)^{aB}	0.7(±0.2)^{abA}	0.2(±0.1)^{bA}	0.8(±0.2)^{aA}
b) Third leaves									
9	3.1(±0.8)^{aA}	2.5(±0.2)^{aA}	1.7(±0.3)^{aB}	3.7(±1.3)^{aA}	2.5(±0.6)^{aA}	3.3(±0.5)^{aA}	-	-	-
11	1.0(±0.4)^{bB}	4.2(±0.6)^{aA}	2.2(±0.8)^{bA}	1.3(±0.3)^{bAB}	3.5(±0.5)^{aA}	2.3(±0.4)^{abA}	2.4(±0.5)^{aA}	2.9(±0.7)^{aA}	2.7(±0.3)^{aA}
13	5.2(±0.8)^{aA}	1.1(±0.4)^{bB}	1.2(±0.1)^{bA}	0.6(±0.1)^{bB}	1.8(±0.4)^{aB}	1.8(±0.4)^{aA}	2.1(±0.6)^{bB}	4.8(±0.3)^{aA}	1.6(±0.4)^{bA}
c) Second Leaves									
6	-	-	-	-	-	-	-	-	-
8	1.7(±0.1)^{aA}	1.6(±0.1)^{abAB}	1.3(±0.1)^{bB}	1.1(±0.0)^{aB}	1.2(±0.1)^{aB}	1.3(±0.0)^{aB}	1.5(±0.1)^{aA}	1.7(±0.2)^{aA}	1.7(±0.1)^{aA}
10	1.6(±0.3)^{aA}	0.4(±0.2)^{bA}	0.3(±0.1)^{bA}	0.5(±0.1)^{aB}	0.3(±0.1)^{aA}	0.3(±0.2)^{aA}	0.5(±0.2)^{aB}	1.2(±0.1)^{aA}	0.8(±0.1)^{aA}
d) Third leaves									
9	-	-	-	-	-	-	-	-	-
11	4.9(±0.6)	-	-	-	-	-	-	-	-
13	3.1(±0.7)^{aA}	0.8(±0.3)^{abA}	0.3(±0.1)^{bA}	0.5(±0.1)^{aB}	0.6(±0.1)^{aA}	0.3(±0.1)^{aA}	0.6(±0.2)^{aAB}	1.0(±0.3)^{aA}	0.6(±0.2)^{aA}

These findings are in agreement with reports on tomatoes cultivars by Nahar and Gretzmacher (2011) who found that root and shoot length response to drought was variety dependent with some varieties having longer roots and others having shorter roots. The root:shoot ratio were higher under water stress in both sorghum varieties, a behavior similar to that shown by most plant species (Khurana and Singh 2000; Nahar and Gretmacher 2011; Sun *et al*. 2011). However, under water stress, there root:shoot ratio was higher for seedlings growing from Susu seeds compared to those of Piper and this can be attributed to their larger mass as previously reported by Khurana and Singh (2000). We found that the total biomass was reduced under water stress in seedlings growing from Piper seeds and this was in agreement with reports in sugar beet by Ober and Luterbacher (2002). Seedlings growing from Susu seeds showed the highest total biomass at 50% FC, a behavior that contrasted that reported by Pimratch *et al*. (2008) where biomass could be reduced up to 13% under 2/3 water availability in the soil. However, this observation could be attributed to its possession of a gene that promotes biomass production under 50% FC.

The second and third LER of both varieties was highest under fully watered conditions and lowest under severe drought. The second leaf elongation rate was higher in seedlings growing from Piper seeds compared to those from Susu because these leaves kept elongating as time passed but remained unfurled. In Susu, the second leaves stopped developing earlier leading to the initiation of a third leaf which explains why the third LER of Susu seedlings were higher (Table I). Therefore, Susu seedlings showed higher vigor than Piper seedlings. Drought stress promoted relative leaf expansion rate in seedlings from Susu seeds but the reverse was true for the second leaves of seedlings from Piper seeds. The results for seedlings from Piper seeds and not those from Susu seed was in agreement with reports by Vurayai *et al.* (2011).

5.4.2 Drought effects on seedlings growth from coated seeds

In both varieties, Stockosorb promoted root length development and this could be attributed to the ability of this hydro-absorber to attracted additional water Gorim and Asch (2012) enabling a greater soil volume to be exploited; an important adaptation in drought spell. Geohumus is known to physically hinder or slow down root development after germination (personal communication) and this could be the reason for the observed poor and slow root development. Therefore, under fully watered conditions, the root length was promoted by Stockosorb but retarded by Geohumus which promoted root growth at 50 % FC. In Piper, the root:shoot ratio under hydro-absorber coatings was lower than those of seedlings from uncoated seeds at all moisture levels (Fig. 1) implying that hydro-absorbers tended to cushion the seedlings during water stress.

In Susu, the root:shoot ratio was highest in seedling growing from Stockosorb seeds at 25% FC and this could be as a result of more resources being channeled into root biomass. Seedlings from Stockosorb coated seeds under fully watered conditions also had the highest root:shoot ratio and this was in agreement with investigations that report that Stockosorb promotes root development (Gorim and Asch 2010). Root and Shoot growth was repressed in seedlings growing from Geohumus seeds compared to the other treatments irrespective of their moisture level although at 25 % FC higher root:shoot ratio was observed; a result that was in agreement with other studies (Nahar and Gretmacher 2011; Sun *et al*. 2011). In Piper, both hydro-absorbers decreased biomass production and this could be attributed to their ability to trap nutrients making them available to the plants (Duong and Asch 2011). In Susu, the presence of Stockosorb promoted biomass production under fully watered conditions and severe drought. Biomass was higher in the drought treatments in seedling growing from Geohumus coated seeds which is possible since leaf development was promoted by this coating. Differences in these 2 varieties could also result from their possession of different genotypic traits.

5.4.3 Drought effects on leaf parameters in seedlings growing from coated seeds

Geohumus promoted second LER better than Stockosorb in seedlings growing from Piper seeds but in the case of the third LER, seedlings from both coatings performed better than those from uncoated seeds. However, Geohumus promoted third LER in seedlings growing from Piper seeds under drought better than Stockosorb and this could be attributed to the presence of additional nitrogen incorporated into Geohumus. The fact that the second leaf expansion rate was lower in seedlings growing from Stockosorb coated Piper seeds imply that those coated with

Geohumus had broader and longer second leaves under drought conditions meanwhile their third leaves under drought stress were longer but folded. Under fully watered conditions, the second LER in seedlings growing from Susu seeds was highest compared to the other treatments meanwhile under drought stress, both coating had higher second LER. Seedling from both uncoated and Stockosorb coated seeds had similar third LER at all moisture levels which was higher than that for seedlings growing from Geohumus seeds. The second RLER was higher in seedlings growing from Geohumus seeds compared to the other treatments but both coating showed increased RLER as drought progressed. This was in contrast with reports by Ober and Luterbacher (2002) and Vurayai *et al.* (2011) that showed that the presence of drought led to a decrease in RLER. Seedlings growing from Geohumus coated seeds under drought showed increased third RLER and. increase in the third RLER under fully watered conditions was also been observed in seedlings growing from Geohumus coated seeds but the reverse was true for the others. The odd behavior effected by Geohumus is not fully understood.

5.4.4 Interaction between drought stress, coatings and varieties

Growth in root length was strongly suppressed by drought in seedlings growing from Geohumus coated seeds compared to those of Susu coated seeds. Stockosorb did not strongly promote root length growth in seedlings growing from Susu seeds compared to those in Piper. The variable behavior of these 2 genotypes agrees with reports by Nahar and Gretzmacher (2011) which showed that the root length response of different cultivar of tomato to increasing drought was variable. The root:shoot ratio of seedlings growing from Stockosorb coated Susu seeds under fully watered conditions was lower than that under 25 % FC but the opposite was true for seedlings growing from Piper seeds; a behavior that could be attributed to genotypic differences. Seedlings growing from Geohumus coated Piper seeds at 50 % FC had the highest root:shoot ratio but this was also true for seedlings growing from Susu seeds at 25 % FC (Fig. 1 & 3 f). The total biomass produced was higher for seedlings growing from Geohumus coated Susu seeds at 25 % FC meanwhile for seedlings growing from Piper seeds; the highest biomass was produced at 50 % FC. However, when both varieties were coated with Geohumus, they produced lower biomass inspite of the moisture level at which they grew. Seedlings growing from Stockosorb coated seeds in both varieties produced more biomass under at 25 % FC compared to those from uncoated seeds. Seedlings growing from Stockosorb coated Susu seeds in contrast to those seedlings growing from Piper seeds, produce more biomass under fully watered conditions compared to the other treatments (Fig. 2h).

The second LER of seedlings growing from both Geohumus and Stockosorb coated seeds was higher in Piper at all moisture levels compared to Susu but in Piper, LER were lower than those of seedlings growing from the uncoated seed and the opposite was true for seedlings growing from Susu seeds. The third LER of Seedlings growing from Stockosorb coated seeds was lower in seedlings growing from Piper seeds than under fully watered conditions. However, the third LER of Seedlings growing from Stockosorb coated seeds were similar at all moisture regimes to those of the Seedlings growing from uncoated seeds meanwhile the third LER of seedlings growing from Piper seeds were lower than those of the Seedlings growing from uncoated seeds under fully watered conditions. However, the third LER Seedlings growing from Geohumus coated Susu seeds were lower than those of seedlings growing from uncoated seeds under fully watered conditions. The leaf expansion rate of the 2 varieties was different under fully watered conditions and drought stress. Seedlings growing from Susu seeds rolled out their leaves earlier with higher RLER compared to those seedlings growing from Piper seeds and as a result, both the second and third leaves of seedlings growing from Piper seeds had lower RLER than those seedlings growing from Susu seeds.

5.4.5 Drought effects on seedling height

The height of both varieties was affected differently by drought. Stockosorb promoted vertical plant growth under fully watered conditions compared to the others. Geohumus suppressed seedling height even though it promoted leaf development and this could be a reason for the very low biomass observed in these seedlings especially in the variety, Piper. Seedlings grow from Piper seeds were shorter than those growing from Susu seeds irrespective of their moisture level. This may be due to the fact that their seed mass was smaller than those of Susu as described for *Albizia procera* by Khurana and Singh (2000). At FC, seedlings growing from uncoated Piper seeds were significantly taller compared to those resulting from coated seeds and this could be attributed to emergence delay observed in seedlings growing from coated seeds (Berdahl and Barker 1980; Gorim and Asch 2011). We observed that drought did not significantly reduce the height of seedlings growth from Piper seeds; this was in contrast with reports by Khurana and Singh (2000) but in agreement with reports by Sun *et al* (2011) where the seedling height was not significantly affected both under drought and fully watered conditions.

5.5 Conclusions

Seedlings coated with the 2 hydro-absorbers as well as the 2 varieties tested responded differently under drought. Seedlings growing from Stockosorb coated seed performed better that those coated with Geohumus during drought. However, Geohumus promoted seedling leaf growth better than Stockosorb. Also, Stockosorb is preferred over Geohumus because Geohumus interacts and traps nutrients which are not readily available for plant growth. However, Geohumus would be preferred in a situation where plant leaves are of interest like for the growth of leafy vegetables. Susu was more susceptible than Piper to drought having significantly higher root:shoot ratio even in the presence of Stockosorb although both varieties showed depressed biomass. However, seedling growth was better in this variety. We therefore conclude that the analysis of the response of seedlings to drought should take into consideration the type of hydro-absorber to be employed in coating and seed variety. Also, further development by seed companies together with researchers is needed to better develop hydro-absorbers available on sale since most of their labeled information has proven inconsistent with reports from trails.

5.6 References

Abdulai, A.L., Parzies, H., Kouressy, M., Vaksmann, M., Asch, F. and Brueck, H. 2012: Yield stability of photoperiod sensitive sorghum (*Sorghum bicolor* L. Moench) accessions under diverse climatic environments. International Journal of Agricultural Research 7(1): 17-32.

Ali, M.A., Jabran, K., Awan, S.I., Abbas, A., Ehsanullah, Zulkiffal, M., Acet, T., Farooq, J. and Rehman, A. 2011: Morpho-physiological diversity and its implications for improving drought tolerance in grain sorghum at different growth stages. Australian Journal of Crop Science 5(3): 311-320.

Berdahl, J.D. and Barker, R.E. 1980: Germination and emergence of Russian wildrye seeds coated with hydrophilic materials. Agronomy Journal 72: 1006-1008.

Borrell, A.K., Jordan, D., Mullet, J., Klein, P., Klein, R., Nguyen, H., Rosenow, D., Hammer, G., Douglas, A. and Henzell, B. 2004: Discovering stay-green drought tolerance genes in sorghum: A multidisciplinary approach. Fourth International Crop Science Congress. September 2004 Brisbane, Australia. p. 1-5

Clark, R.B. 1982: Nutrient solution growth of sorghum and corn in mineral nutrition studies. Journal of Plant Nutrition 5 (8): 1039-1057.

Duong, V.N. and Asch, F. 2011: Effects of soluble salts on water holding capacity of hydrophilic polymers: A case study on Geohumus. Tropentag 2011. October 5-7, Bonn Germany. p. 512.

FAO, 1996: The world sorghum and millet economies: facts, trends and outlook. http://www.fao.org/documents/en/detail/22369 Assessed 07.11.2011.

Gorim, L. and Asch, F. 2012: Effects of composition and share of seed coatings on the mobilization efficiency of cereal seeds during germination. Journal of Agronomy and Crop Science 198(2): 81-91.

Gorim, L. and Asch, F. 2010: Effects of hydrophilic coatings on the mobilization of endosperm reserves in seedlings of spring barley (*Hordeum vulgare* L. maltasia). World food system- A contribution from Europe. Tropentag 2010. Zurich, Switzerland. S. 337.

Hassan, Z.A., Young, S.D., Hepburn, C. and Arizal, R. 1990: An evaluation of urea-rubber matrices as slow-release fertilizer. Fertilizer Research 22: 63-70.

Hlavinka, P., Trnka, M., Semeradova, D., Dubrovsky, M., Zalud, Z. and Mozny, M. 2009: Effect of drought on yield variability of key crops in Czech Republic. Agricultural and Forest Meteorology 149: 431-442.

Pimratch, S., Jogloy, S.P.,Vorasoot, N., Toomsan, B., Patanothai, A. and HolbrooK, C.C. 2008: Relationship between biomass production and nitrogen fixation under drought stress conditions in peanut genotypes with different levels of drought resistance. Journal of Agronomy and Crop Science 194: 15-25.

Kaufman, G. 1991: Seed coating: A tool for stand establishment; a stimulus to seed quality. Horticulture Technology 1(1): 98-102.

Khurana, E. and Singh, J.S. 2000. Influence of seed size on seedling growth of *Albizia procera* under different soil water levels. Annals of Botany 86: 1185-1192.

Marteau, R., Sultan, B., Moron, V., Alhassane, A., Baron, C., Traoré, S.B. 2011: The onset of the rainy season and farmers' sowing strategy for pearl millet cultivation in southwest Niger. Agricultural and Forest Meteorology 151 (10): 1356-1369.

Mikkelsen, R.L., Behel Jr., A.D. and Williams, H.M. 1993: Addition of gel forming hydrophilic polymers to nitrogen fertilizer solutions. Fertilizer Research 36: 55-61.

Mutava, R.N., Prasad, P.V.V., Tuinstra, M.R., Kofoid, K.D. and Yu, J. 2011: Characterization of sorghum genotypes for traits related to drought tolerance. Field Crops Research 123: 10-18.

Nahar, K. and Gretzmacher, R. 2011: Response of shoot and root development of seven tomato cultivars in hydroponic systems under water stress. Academic Journal of Plant Sciences 4(2): 57-63.

Nour, A.E.M., Weibel, D.E. and Todd, G.W. 1978: Effect of repeated drought periods on the survival of sorghum seedlings. Agronomy Journal 70 (3): 509-510.

Ober, E.S. and Luterbacher, M.C. 2002. Genotypic variation for drought tolerance in *Beta vulgaris*: Annals of Botany 89: 917-924.

Patil, S.L. 2007: Performance of sorghum varieties and hybrids during post-rainy season under drought situations in vertisols in Bellary, India. Journal of SAT Agricultural Research 5(1): 1-3.

Powell, A.A. and Matthew, S. 1988: Seed treatment: development and Prospect. Outlook on Agriculture 17: 93-103.

Sabadin, P.K., Malosetti, M., Boer, M.P., Tardin, F.D., Santos, F.G., Guimarães, C.T., Gomide, R.L., Andrade, C.L.T., Albuquerque, P.E.P., Caniato, F.F., Mollinari, M., Margarido, G.R.A., Oliveira, B.F., Schaffert, R.E., Garcia, A.A.F., van Eeuwijk, F.A. and Magalhaes, J.V. 2012: Studying the genetic basis of drought tolerance in sorghum by managed stress trials and adjustments for phenological and plant height differences. Theoretical and Applied Genetics 124 (8): 1389-1402

Scott, J.M., Jessop, R.S., Steer, R.J. and Mclachlan, G.D. 1987: Effects of nutrients seed coating on the germination of wheat and oats. Fertilizer Research 14: 205-217.

Smith, R.L., Hoveland, C.S. and Hanna, W.W. 1989: Water stress and temperature in relation to seed germination of pearl millet and sorghum. Agronomy Journal 81 (2): 303-305.

Takele, A. 2000: Seedling emergence and growth of sorghum genotypes under variable soil moisture deficit. Acta Agronomica Hungerica 48 (1): 95-102.

van de Giesen, N., Liebe, J. and Jung, G. 2010: Adapting to climate change in the Volta Basin, West Africa. Current Science 98 (8): 1033-1037.

Volaire, F. 2003: Seeding survival under drought differs between an annual (*Hordeum vulgare*) and a perennial grass (*Dactylis glomerata*). New Phytologist 160: 501-510.

Vurayai, R., Emongor, V. and Moseki, B. 2011: Effect of water stress imposed at different growth and development stages on morphological traits and yield of bambara groundnuts (*Vigna subterranean* L. Verdc). American Journal of Plant Physiology 6 (1): 17-27.

Xu, Z., Zhou, G. and Shimizu, H. 2009: Are plant growth and photosynthesis limited by pre-drought following rewatering in grass? Journal of Experimental Botany 60(13): 3737-3749.

6 General conclusions and recommendations

The presence of additional coatings especially hydro-absorber coatings on barley, rye and wheat seeds reduced germination rates but this reduction depended on the coat share and the cereal coated. Seedlings from seeds with coat share that were > 75% of their grain weight performed better having better root development compared with those from uncoated seeds. The seed within hydro-absorber coated seeds had higher moisture contents and in the case of wheat, this may have resulted in flooding situations within the seed. The amount of oxygen in the embryo of coated barley, rye and wheat seeds was reduced and this together with the imbibed moisture influenced how reserves were mobilized. Mobilization within coated seeds happened more efficiently resulting in seedling with higher biomass compared to those that were uncoated. This efficient use of stored reserve resulted from a preference for energy conserving metabolic pathways during sugar metabolism. Surprisingly, all three cereals mobilized sucrose differently during phase I and III of germination. Although seedling health improved when coatings were present in barley, rye and wheat, when both Geohumus and Stockosorb were assessed in 2 sorghum varieties, Susu and Piper under 2 drought levels, they did not significantly influence seedling growth although Geohumus promoted leaf growth. I recommend that further investigation on the amount of moisture in combination with oxygen that triggers germination be carried out because the question of why some seeds die while others germinate has not yet been answered. Furthermore, oxygen profile for the entire embryo needs to be mapped outs as well as the scutellum and aleurone layer; a study that needs to be done in conjunction with the assessment of enzymes involved during starch and sucrose breakdown. Further development of hydro-absorber products such that they show better interactions with nutrient solutions and seed coats is warranted. Finally, germination of seed is a complex event that is not yet fully understood but the development of micro-sensor enabling in situ measurements of oxygen within seeds has opened up another window of opportunity to better understand the processes involved.

7 Additional references

Abu-Ghannam, N. and McKenna, B. 1997: Hydration kinetics of kidney beans (*Phaseolus vulgaris* L.). Journal of Food Science 62: 520-523.

Arancon, G.N., Edwards, A.C., Lee, S. and Byrne, R. 2006: Effects of humic acids from vermicomposts on plant growth. European Journal of Soil Biology 42: 65-69.

Asatsuma, S., Sawada, C., Itoh, K., Okito, M., Kitajima, A. and Mitsui, T. 2000: Involvement of α -amylase I-1 in starch degradation in rice chloroplasts. Plant and Cell Physiology 46: 858-869.

Asik, B.B., Turan, A.M., Celik, H. and Katkat, V.A. 2009: Effects of humic substances on plant growth and mineral nutrients uptake in wheat (*Triticum durum* cv. Salihi) under conditions of salinity. Asian Journal of Crop Science 1: 87-95.

Bai, Z.G., Dent, D.L., Olsson, L. and Schaepman, M.E. 2008: Global Assessment of Land Degradation and Improvement 1. Identification by remote sensing Report 2008/01, ISRIC – World Soil Information, Wageningen, prepared for the FAO executed Land degradation assessment in drylands project.Bundesprogramm Ökologischer Landbau, Projekt 02OE184, Dr. Reiner Schrage; Terhoeven-Urselmans, A. (Author), (2002/04), Landwirtschaftskammer Nordrhein-Westfalen,Pflanzenschutzdienst Bonn, Bundesprogramm Ökologischer Landbau: „Erprobung und Vergleich von Pflanzenstärkungsmitteln an Zierpflanzen im Freiland“, Bonn Germany.

Cooper, R.J., Lui, C. and Fisher, S.D. 1998: Influence of humic substances on rooting and nutrient content of creeping bentgrass. Crop Science 38: 1639-1644.

Hess, R.J. and Carman, J.G. 1993: Normalizing development of cultured *Triticum aesticum* L. embryos. I. Low oxygen tensions and exogenous ABA. Journal of Experimental Botany 44: 1067-1073.

Hüttermann, A., Orikiriza, L.J.B. and Agaba, H. 2009: Review: Application of superabsorbent polymers for improving the ecological chemistry of degraded or polluted lands. Clean 37(7): 517-526.

Maredia, M.K., Byerlee, D. and Pee, P. 2000: Impacts of food crop improvement research: Evidence from sub-Saharan Africa. Food Policy 25: 531–559.

Meyer, C., Steudle, E. and Peterson, C. A. 2007: Patterns and kinetics of water uptake by soybean seeds. Journal of Experimental Botany 58: 717-732.

Mikkelsen, R.L., Behel Jr, A.D. and Williams, H.M. 1993: Addition of gel-forming hydrophilic polymers to nitrogen fertilizer solutions. Fertilizer Research 36:55-61.

Nardi, S., Pizzeghello, D., Muscolo, A. and Vianello, A. 2002: Physiological effects of humic substances on higher plants. Soil Biology and Biochemistry 34: 1527-1536.

Nha, D.V. 2012: Enviromental effects on physical properties of Geohumus and effects of its application on drought responses in maize. Dissertation. Faculty of Agricultural Sciences. Universtät Hohenheim, Stuttgart. Germany.

Okamoto, K. and Akazawa, T. 1980: Enzymic mechanism of starch breakdown in germinating rice seeds. De novo synthesis of β-amylase. Plant Physiology 65: 81-84.

Patil, S.L. 2007: Performance of sorghum varieties and hybrids during post-rainy season under drought situations in Vertisols in Bellary, India. Journal of SAT Agricultural Research 5(1): 1-3.

Porter, G.N. and Wareing, F.P. 1974: The role of the oxygen permeability of the seed coat in the dormancy of seed of *Xanthium pennsylvanicum* Wallr. Journal of Experimental Botany 25(86): 583-594.

Quarrie, A.S., Stojanovic, J. and Pekic, S. 1999: Improving drought resistance in small-grained cereals: A case study, progress and prospects. Plant Growth Regulation 29: 1-21.

Rosenzweig, C. and Hillel, D. 1998: Climate Change and The Global Harvest: Potential Effects of the Greenhouse Effect on Agriculture. Oxford University Press. New York. 324 pp.

Schneider, A. and Renault, P. 1997: Effects of coatings on seed imbibition: I. Model estimated of water transport coefficient. Crop Science 37: 1841-1849.

Scott, S.J., Jones, R.A. and Wiliams, W.A. 1984: Review of data analysis methods for seed germination. Crop Science 24: 1192-1199.

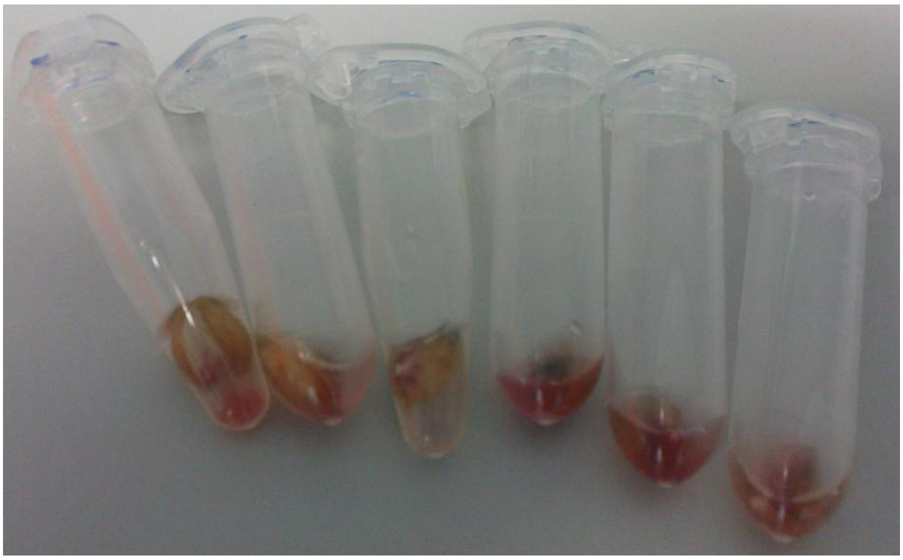
Sopade, P.A. and Obekpa, J.A. 1990: Modeling water absorption in soybean, cowpea and peanuts at three temperatures using Peleg's equation. Journal of Food Science 55: 1084-1087.

Zimmermann, P and Mathias, A. 2002. Bericht zum Versuch mit dPflanzenstärkungsmittel Biplantol agrar, Hochschule Wädenswil, Grüental, Postfach 335, CH-8820 Wädenswil, Switzerland.

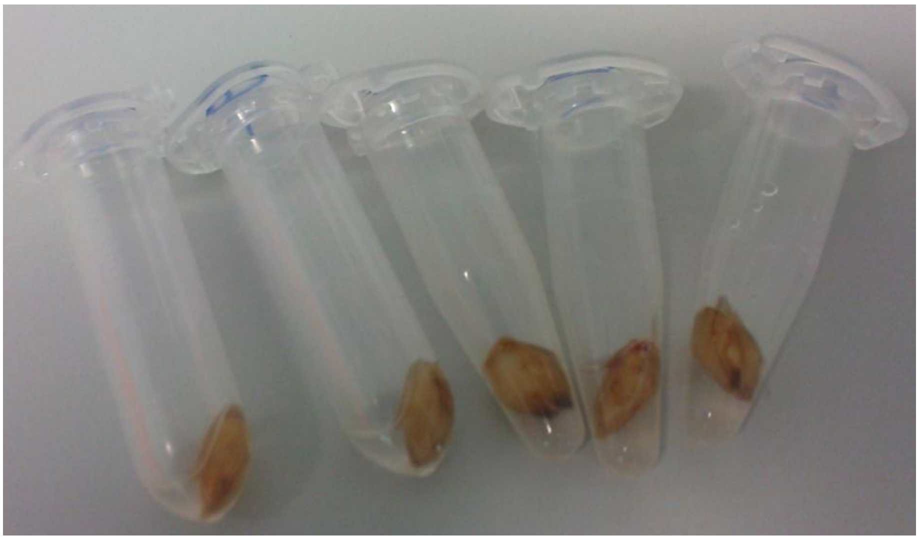
8 Appendex

Tetrazolium test for seed viability

Viable seeds: prominent pink color at the embryo



Dead seeds: no color change



Imbibition and subsequent germination

Uncoated Cereals

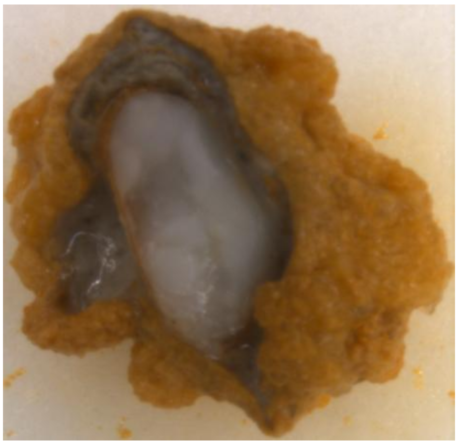
Barley

Rye

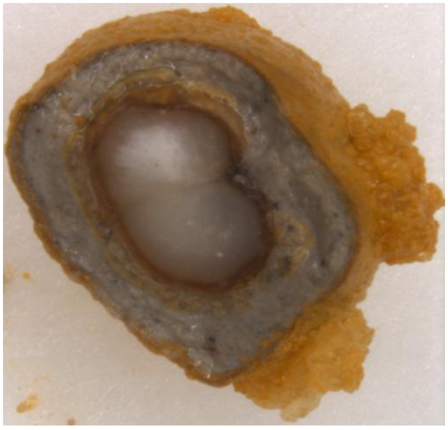
Wheat

Coated cereals

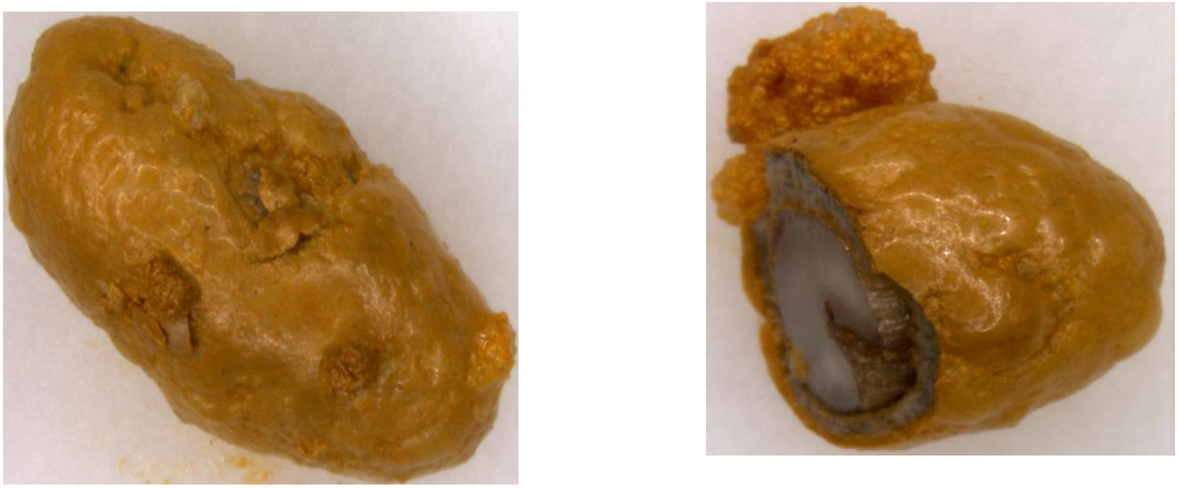
Barley coated with Stockosorb



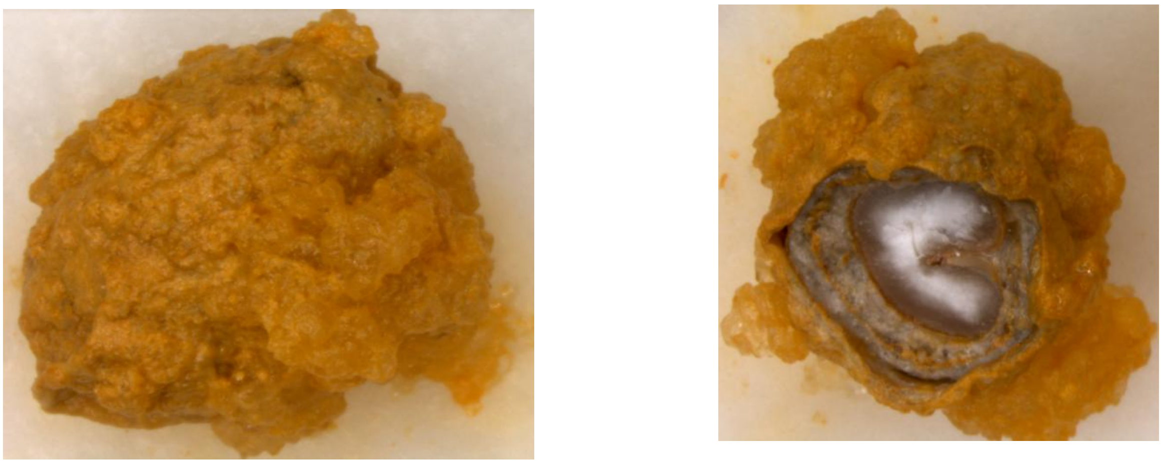
Barley coated with ALL [combination of Stockosorb, Biplantol and humic acids]



Rye coated with hydro-absorber Stockosorb



Wheat coated with the hydro-absorber, Stockosorb



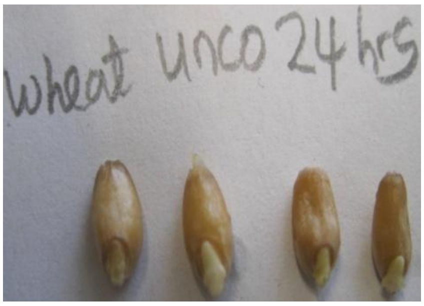
Germination

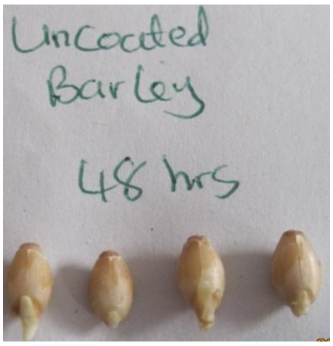
Kernels from coated seeds during the germination process

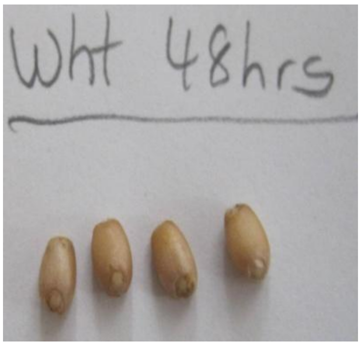
Barley **Rye** **Wheat**

Uncoated seeds during the germination process

Barley **Rye** **Wheat**



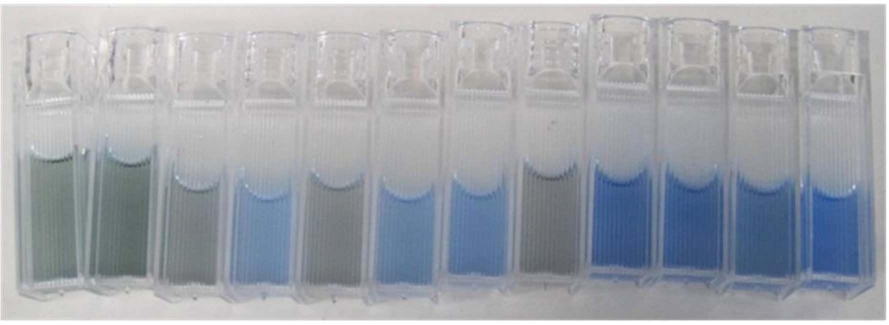




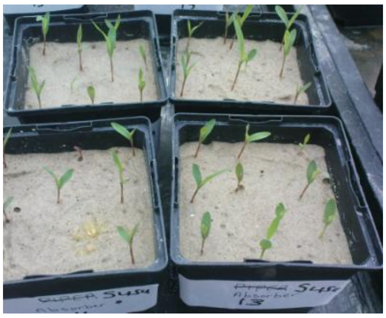
Colored product in test tubes placed water bath during the measurement of sugars and starch


11/01/2013

Colored product in semi-micro cuvettes during the measurement of protein; to be read in the spectrophotometer

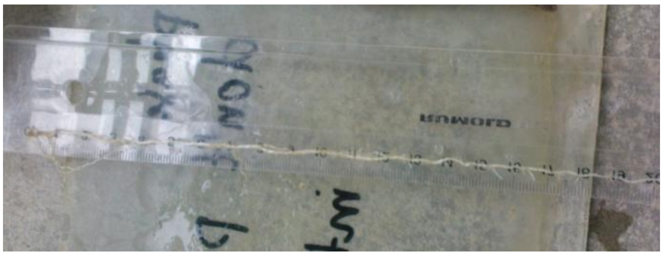


Sorghum grown in the greenhouse in pots containing sand



Pot: Sorghum

Measuring sorghum root length with ruler



Sorghum seeds coated with Geohumus show bizaar behavior; shoots without seeds which later grew after the coats were washed off and shoot plus remaining grain placed on moist petri dishes

Declaration of originality

I hereby declare that this doctoral thesis is independently written by myself. In addition, I confirm that no sources than those specified in the thesis have been used. I assure that this thesis, in the current or similar format, has not been submitted to any other institution in order to obtain a Ph.D. or any other academic degree.

Ich erkläre hiermit, dass ich diese Dissertation selbständig angefertigt habe. Es wurden nur die im Literaturverzeichnis aufgeführten Hilsmittel benutzt und fremdes Gedankengut als solches kenntlich gemacht. Ich versichere, dass ich diese Arbeit in gleicher oder ähnlicher Form noch keiner anderen Institution zur Prüfung vorgelegt habe.

Date: 16/1/2014, Hohenheim   Linda Yuya Gorim