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Hydropriming and seed additives in soybean as measures of improving early plant development

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"Failure is success in progress"

Albert Einstein

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Summary

Soybean production is increasing because it provides high-quality protein for human consumption and animal feed, especially for monogastric animals. Successful soybean production in the temperate climate of central Europe is challenged by late field emergence with a short vegetative growth period. Germination can be improved by seed hydropriming, a process of starting the first stage of germination under controlled conditions to reach a certain point then the process is stopped. If these seeds are sown later, the germination process is accelerated. The technique is used commercially in different crops for example, in sugar beet and vegetables. Another option to improve plant growth is the use of seed additives, namely plant growth-promoting bacteria (PGPR) and arbuscular mycorrhizal fungi (AMF). They mainly improve the nutrient supply and can improve yield in various crops. In addition, isoflavonoids, for instance, genistein play a crucial role in the process of symbiosis development between *Bradyrhizobia* and soybean and can be used for co-inoculation, leading to improved symbiotic nodulation under low soil temperatures. This study aimed to find a hydropriming protocol suitable for improving soybean emergence under cold growing conditions and to test commercially available seed additive products.

Five early maturing soybean varieties commonly used by German farmers were selected. Seeds were first hydroprimed in distilled water for 4, 8, 12, and 16 hours, followed by air drying for 25 hours at room temperature, while no hydropriming served as a control. Seed size increase and water uptake were recorded. In the pot experiment done with field soil and sand, carried out in climate chambers at 9, 12, 15, and 18°C, the time to emergence was studied after the above-mentioned hydropriming durations. In another pot experiment at 15°C, the effects of a PGPR product, an AMF product, and genistein as seed additives on emergence and early plant development were studied. In a subsequent two-year field study, the best options for hydropriming and seed additives were tested with three soybean varieties. Various plant characteristics relating to emergence, root and shoot development, and nodule development were recorded during the growing season. Plant development was also measured at harvest, including the number of pods per plant, the height of the first pod above ground, yield, and N content of beans.

Hydropriming treatments reduced the number of seeds germinated and emerged, without improving the overall speed of germination. Only under 12°C the seed emerged faster after 12 h of hydropriming compared to the control. The seed additives under study did not significantly improve emergence or early growth, but the AMF product tended to favour nodule development. Under field conditions, after hydropriming fewer seeds emerged and the overall plant development and yield was not improved. The addition of the AMF product did not influence plant development and yield.

All experiments showed challenging results for hydropriming. Contrary to the initial hypothesis, hydropriming did not improve germination and emergence but reduced the germinability of the soybean seeds under study. Further research is needed to develop a priming protocol suitable for the soybean varieties used in Central Europe, either by aerating the seeds or using other priming techniques such as osmopriming, solid matrix priming, or biopriming. The AMF, PGPR, and genistein products under study also showed no improvement in plant development or yield. These results are also contrary to the initial hypothesis, but the validity of these results is limited by the number of products and replications in this study. Further research should focus on seed additives for soybeans under cold conditions. At this stage further research on hydropriming and seed additives for soybean is needed before these techniques can be transferred to practical farming.

Zusammenfassung

Die Sojaproduktion nimmt zu, weil sie hochwertiges Eiweiß liefert, sowohl für den menschlichen Verzehr als auch als Futtermittel, insbesondere für Monogastrier. Der erfolgreiche Sojabohnenanbau im gemäßigten Klima Mitteleuropas wird durch den späten Feldaufgang mit einer kurzen vegetativen Wachstumsperiode erschwert. Die Keimung kann durch Hydropriming des Saatguts verbessert werden. Dabei wird das erste Keimstadium unter kontrollierten Bedingungen bis zu einem bestimmten Punkt eingeleitet, dann wird der Prozess gestoppt. Werden diese Samen später ausgesät, ist der Keimprozess beschleunigt. Diese Technik wird kommerziell bei verschiedenen Kulturen eingesetzt, beispielsweise bei Zuckerrüben und Gemüse. Eine weitere Möglichkeit zur Verbesserung des Pflanzenwachstums besteht in der Verwendung von Saatgutzusätzen, vor allem von pflanzenwachstumsfördernden Bakterien (plant growth promoting bacteria, kurz PGPR) und arbuskulären Mykorrhizapilzen (arbuscular mycorrhizal fungi, kurz AMF). Sie verbessern vor allem die Nährstoffversorgung und können den Ertrag verschiedener Kulturpflanzen steigern. Darüber hinaus spielen Isoflavonoide, z. B. Genistein, eine entscheidende Rolle bei der Entwicklung der Symbiose zwischen *Bradyrhizobia* und Sojabohnen und können für die Koinokulation verwendet werden, was zu einer verbesserten symbiotischen Nodulation bei niedrigen Bodentemperaturen führt. Ziel dieser Studie ist es, ein Hydropriming-Protokoll zu finden, das geeignet ist, den Aufgang von Sojabohnen unter kalten Wachstumsbedingungen zu verbessern und kommerziell erwerbliche Saatgutzusatzprodukte zu testen.

Es wurden fünf früh-abreifende Sojabohnensorten ausgewählt, die in der Praxis im deutschen Sojaanbau verwendet werden. Die Bohnen wurden zunächst 4, 8, 12 und 16 Stunden lang in destilliertem Wasser hydrogeprimt und anschließend 25 Stunden lang bei Raumtemperatur an der Luft getrocknet. Die Zunahme der Saatgutgröße und die Wasseraufnahme wurden aufgezeichnet. In dem Gefäßversuch mit Feldboden und Sand, durchgeführt in Klimakammern bei 9, 12, 15 und 18°C, wurde die Zeit bis zum Auflaufen nach den oben genannten Hydropriming-Dauern untersucht. In einem weiteren Gefäßversuch bei 15°C wurden die Auswirkungen eines PGPR-Produkts, eines AMF-Produkts und von Genistein als Saatgutzusatz auf den Pflanzenaufgang und die frühe Pflanzenentwicklung untersucht. In einer anschließenden zweijährigen Feldstudie wurden die besten Optionen für Hydropriming und Saatgutzusatzmittel mit drei Sojabohnensorten getestet. Während der Vegetationsperiode wurden verschiedene Pflanzenmerkmale in Bezug auf den Aufgang, die Wurzel- und Sprossentwicklung sowie die Entwicklung der Knöllchen erfasst. Die Pflanzenentwicklung wurde auch bei der Ernte erfasst, einschließlich der Anzahl der Hülsen pro Pflanze, der Höhe der ersten Hülse über dem Boden, des Ertrags und des Stickstoffgehalts der Bohnen.

Die Hydropriming-Behandlungen verringerten die Anzahl der gekeimten und aufgegangenen Samen, ohne die Gesamtgeschwindigkeit der Keimung zu verbessern. Nur unter 12°C keimte das Saatgut nach 12 Stunden Hydropriming im Vergleich zur Kontrolle schneller auf. Die untersuchten Saatgutzusätze verbesserten weder den Aufgang noch das frühe Wachstum signifikant, aber das AMF-Produkt begünstigte tendenziell die Entwicklung der Knöllchen. Unter Feldbedingungen gingen nach dem Hydropriming weniger Samen auf, und die Pflanzenentwicklung und der Ertrag wurden insgesamt nicht verbessert. Die Zugabe des AMF-Produkts hatte keinen Einfluss auf die Pflanzenentwicklung und den Ertrag.

Alle Versuche ergaben problematische Ergebnisse für das Hydropriming. Entgegen der ursprünglichen Hypothese verbesserte das Hydropriming nicht die Keimung und den Aufgang, sondern verringerte die Keimfähigkeit der untersuchten Sojabohnensamen. Weitere Forschungsarbeiten sind erforderlich, um ein für die in Mitteleuropa verwendeten Sojabohnensorten geeignetes Priming-Protokoll zu entwickeln, entweder durch Belüftung des Saatguts oder durch die Anwendung anderer Priming-Techniken wie Osmopriming, Festmatrix-Priming oder Biopriming. Die getesteten AMF-, PGPR- und Genistein-Produkte zeigten ebenfalls keine Verbesserung der Pflanzenentwicklung oder des Ertrags. Diese Ergebnisse stehen ebenfalls im Widerspruch zu der ursprünglichen Hypothese, aber die Gültigkeit dieser Ergebnisse ist durch die Anzahl der Produkte und Wiederholungen in dieser Studie begrenzt. Weitere Forschungsarbeiten sollten sich auf Saatgutadditive für Sojabohnen unter kalten Bedingungen konzentrieren. Zum gegenwärtigen Zeitpunkt sind weitere Forschungsarbeiten zum Hydropriming und zu Saatgutzusätzen für Sojabohnen erforderlich, bevor diese Verfahren auf den praktischen Anbau übertragen werden können.

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1 Introduction

Soybeans are interesting from both a nutritional and agronomic point of view. It is therefore not surprising that soybean production is increasing, as is human consumption. However, European production faces certain problems in terms of optimal germination conditions.

This chapter describes the benefits of soybean, its historical and current use in Europe, and the problems of soybean production under European growing conditions. An introduction to seed priming and seed additives is also given, as these may be used to address these challenges.

1.1 Nutritional value of soybean

Soybean (*Glycine max* L. MERR.) is a major contributor to global high-quality protein production, suitable for both human consumption and animal feed. Soybean wholemeal covers 70% of the European demand for protein-rich feed raw materials (Bernet et al., 2016). Essential amino acids, such as Lysine, Methionine, Cysteine and Tryptophan, are vital for humans and monogastric animals - but are often in short supply. Soybean provides a valuable source of these nutrients. Therefore, soybean has the potential to supplement animal-based products in human diets and offer high-quality animal feed.

Essential amino acids must be obtained from the diet to supply the organism's demand. As described in "Liebig's Law of the Minimum" the amino acid with the lowest supply determines the proportion of absorbed protein from the food/feed, which is also expressed as the biological value of protein (Schlieper, 1990). Soybean protein possesses a high biological value that is comparable to animal protein. The additional advantage is that it contains all essential amino acids and is lower in fat content and cholesterol-free (Engelbert, 2017). Soybeans and their derivatives, for example, tofu and meat substitutes, offer valuable alternatives to conventional animal protein sources for human consumption.

The management of feed ratio for pigs and poultry relies on essential amino acids. The amino acid composition determines satisfactory nutrition for these animals. The protein value can be enhanced by merging soybean and alternative protein sources (Weindl and Bellof, 2016). Soybean is a prevalent protein source for cattle and dairy cows, mostly when supplementing a maize-based ratio (Stopp et al., 2013). Nonetheless, this idea raises doubts concerning environmental concerns.

Before consumption or feeding, soybeans should undergo a heating process, such as toasting, to deactivate antinutritive compounds including trypsin inhibitors (Bernet et al., 2016). Although farmers

often possess adequate facilities to include soybeans in their crop rotation, they still need to obtain or arrange for equipment to heat the beans before use.

1.2 Agricultural value of soybean

Glycine max (L.) MERR. is an annual self-pollinating crop. It has a high oil content of 18-22% and a protein content of around 40% (Jürgen Recknagel, 2010). The seeds are oval-shaped, and the commercial soybean's seed coat is yellow (Purcell et al., 2014), whereas the hilum's colour varies depending on the soybean variety (Schuster, 1998).

Soybeans develop an extensive root system that deeply penetrates the soil (Schuster, 1998). Nodules on the plant's main and lateral roots are formed when *Bradyrhizobium japonicum* is present in the soil or when the seeds are inoculated with this specific bacterium. Factors such as cultivar, plant density, and environmental conditions lead to variations in the height of aboveground biomass and the number of branches. The amount of nitrogen obtained through the symbiosis with *Bradyrhizobia* varies. Under ideal conditions, soybeans can fix up to 300 kg of nitrogen per hectare (Keyser and Li, 1992). However, it is important to carefully consider soybean cultivation practices for optimal nitrogen use efficiency in agriculture.

Compared to other legumes, soybeans leave less nitrogen for subsequent crops and can even act as a net consumer of soil nitrogen (Oberson et al., 2007; Binacchi et al., 2023). Values for the proportion of nitrogen fixation in soybeans through biological means vary in the existing literature. Ciampitti and Salvagiotti (2018) discovered a higher nitrogen imbalance when seed yield and nitrogen content were greater than 370 kg N ha^{-1}. They emphasised that belowground biomass is usually not accounted for nitrogen balances in most studies.

Organic farming requires the integration of leguminous crops due to the limited nitrogen availability. The use of leguminous plants in conventional farming diminishes the energy-intensive demand for mineral nitrogen fertilisers, which is environmentally advantageous. Furthermore, it promotes crop rotation variability that enhances agrobiodiversity, improves soil fertility, and expands flower growth for bees and insects (Bundesinformationszentrum Landwirtschaft, 2021).

For farmers, implementing a diverse crop rotation is a means of yield stability (Macholdt et al., 2020) and therefore reduces economic risk. Given that climate patterns are unforeseeable before planting season, farmers are unable to predict the conditions their crops will be exposed to. Whilst a restricted crop rotation or monoculture can leave the entire crop exposed to a single environmental factor, a variety of crops can alleviate the risk of complete failure. Soybean is a lucrative agricultural commodity and is self-compatibility in contrast to other legumes.

1.3 Soybean Production in Europe

1.3.1 History

Soybeans originated in China/East Asia (Schuster, 1998). In 1875, Professor F. Haberlandt commenced field trials in Europe to introduce soybean as a new crop (Aoyagi, 2020). Haberlandt made several significant discoveries, including the nitrogen fixation of soybean, improved digestibility of soy protein after heat treatment, and the influence of day length on flowering (Rohrer et al., 2018). Although his work did not lead to the establishment of large-scale soybean production in Europe, his expertise was used by the United States, which began field research in 1879 that culminated in the methodical cultivation of soybeans (Rohrer et al., 2018).

During both Worl Wats, soy flour and soy milk were extensively used by both military personnel and civilians in Germany (Shurtleff and Aoyagi, 2007). Starting in 1933, Germany embarked on extensive soybean cultivation in Eastern Europe, encompassing regions like Romania, Bulgaria, Hungary, Yugoslavia, and Austria. It reached its production climax in 1941 with 125,000 tonnes (Shurtleff and Aoyagi, 2007). Following 1945, soybean imports into Germany rose notably, with most of the importation originating from the United States of America. After 1972, Brazil and Argentina also began exporting soybeans to Europe. Shurtleff and Aoyagi described the adaptation of soybean in Europe as follows: in 1980 Europe imported 17 million tonnes of soybean and soy oil is a primary ingredient in margarine and oils for cooking and salad, while the defatted soybean meal was commonly used as cheap livestock feed.

In 2016, global soybean production reached 335 million tonnes on 122 million hectares (FAO, 2023), with the largest production in the United States, Brazil, and Argentina. Only two per cent of the total soybean production is used for direct human consumption, while the remaining 98% is subjected to industrial processing that results in the production of soy oil (20%) and soy meal (80%) destined for use as animal feed (Bundesanstalt für Landwirtschaft und Ernährung, 2020). Half of the European soybean crop was grown in Ukraine in 2016 (Bernet et al., 2016). Due to higher demand for protein feed than domestic production, 60% of plant-based protein is imported (Bernet et al., 2016), primarily from South America and the USA. The high concentration of soybean production in these regions has led to a range of environmental issues, including the direct or indirect conversion of forests into soybean farmland, the use of genetically modified soybean seeds, and extensive herbicide use (Bundesanstalt für Landwirtschaft und Ernährung, 2020).

1.3.2 Recent increase

In 2021, soybeans were grown on 933,000 hectares of land in the European Union (EU), representing a two-fold rise over the previous decade (Bundesinformationszentrum Landwirtschaft, 2021). Italy, France and Romania were the main soybean producers with cultivation on 269,000, 156,000 and 150,000 hectares, respectively.

The extensive programme "Expansion of soybean cultivation in Germany by means of adaptation through breeding and optimisation of crop production and processing technology" was conducted between 2011 and 2013. Several German institutions worked together to examine and improve the soybean value chain, involving research, extension services and soybean processors (Wilbois et al., 2014). In 2013, grain legumes were added to the "Greening" strategies of the Common Agricultural Policy (CAP) by the EU, which also aided in boosting soybean production. The area used to grow soybeans in Germany increased from 4,500 ha in 2011 (Jürgen Recknagel, 2022) to 15,800 ha in 2016 and then to 46,100 ha in 2023 (Figure 1) (Eurostat, 2023)

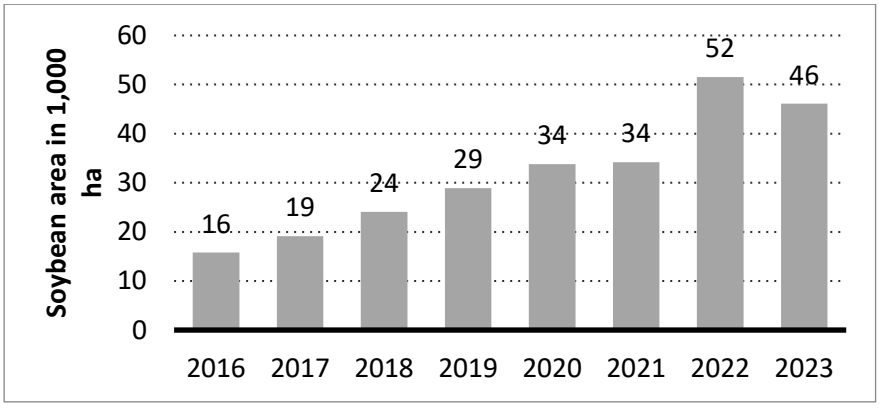

Figure 1 Soybean acreage in Germany from 2016 to 2023. Source Eurostat (2023).

Furthermore, soybean processing and production for meat substitutes in Germany increased by 39% in 2020 compared to 2019 (Bundesinformationszentrum Landwirtschaft 2021). However, it is important to note that most of the soybean produced in Germany is utilised for animal feed. with 46% for on-farm feeding and 42% for oil production for industrial use, soybean cake, a by-product of oil processing, is utilised for animal feed (Bundesinformationszentrum Landwirtschaft, 2021). In 2021, approximately 20 mobile and stationary facilities for soybean toasting were available to farmers in Germany (Bundesinformationszentrum Landwirtschaft, 2021). These facilities permit farmers to process their soybeans for animal feed.

1.3.3 Challenges in Germany

Soybean production is challenging in Germany and other parts of Europe due to the lower temperatures and shorter vegetative growth periods compared to major soybean-producing regions

like Brazil. The optimal temperature for soybean germination is around 30°C (Bachteler, 2016). Additionally, soybeans are short-day plants and flowering is triggered by periods of darkness (Purcell et al., 2014). For European soybean production, North American or Canadian varieties are suitable: they are adapted to colder growing conditions and have minimal response to photoperiods with short days, are day-neutral, or have long-day characteristics (Schuster, 1998). Most of the breeding lines used in Central Europe are thus closely related to soybeans from Canada and Switzerland (Hahn and Wuerschum, 2014). Breeding objectives in Europe were primarily focused on adapting soybean varieties to the region's climatic conditions, later also on quality for tofu production (Klaiss et al., 2020). In recent years, breeding for weed tolerance evolved, using "Artificial Weeds" in the field to select soybean varieties that can successfully compete against these weeds (Horneburg et al., 2017; Klaiss et al., 2020).

A notable constraint to soybean cultivation is water availability. The soil's water reservoir in spring is crucial for seed emergence (Schmidt et al., 2019). After sowing, seeds require adequate water to germinate. Seed size doubles and moisture content increases to 50% within 24 hours after sowing (Purcell et al., 2014). During the flowering stage in midsummer, soybean plants require sufficient water (Schmidt et al., 2019), a lack of water can lead to a reduction in the number of pods in soybeans (Liu et al., 2004), which has also been observed in other grain legumes (Nadeem et al., 2019). Irrigation during this time could be economically advantageous.

In the course of breeding procedures, the maturity groups of soybeans were defined, reaching from ‘0000’ to 8, which describes how long the plants need to reach maturity. Recknagel and Imgraben (2013) proposed a vegetation period of soybeans in Germany of 150 to 180 days, corresponding to maturity group ‘000’ to ‘00’, depending on the field site and variety. Earlier maturity groups, such as ‘0000’, need less time and can cope with lower temperatures in the growing season compared to ‘000’ and ‘00’. However, this usually comes along with reduced yields.

Successful soybean production depends on choosing the appropriate maturity group and variety. The decision depends, besides the production goals (animal or human consumption) (Sobko et al., 2020) on the above-discussed environmental factors. For Germany a map incorporating solar radiation, precipitation, soil quality and temperature was developed, to show where soybean cultivation is possible, showing that in large parts soybean production conditions are good or sufficient (Rossberg and Recknagel, 2017).

Harvesting of soybeans occurs between mid-September and mid-October, with any delays leading to a higher moisture content that can prevent proper drying for harvest (Klaiss et al., 2020). Soybeans should ideally have a water content ranging from 13% to 16%. Drier seeds are more susceptible to

damage, and wet seeds necessitate drying to reach 11.5 to 13% for optimal storage, which leads to additional expenses (Bernet et al., 2016).

As soybean is not native to Europe, *B. japonicum* must be introduced to soil or seeds to enable biological nitrogen fixation (Recknagel and Messmer, 2015). This is typically achieved through seed inoculation directly before sowing (Klaiss et al., 2020). Numerous products exist on the market that may be used for seed inoculation. However, these products differ greatly in their efficacy (Wächter et al., 2013). Furthermore, a soybean variety-product interaction was shown (Zimmer et al., 2016). To evaluate nodule functionality, it is recommended that roots are excavated from the soil at the time of maximum biomass growth (usually around mid-July) to observe the activity and quantity of nodules - when active, the inner part appears red (Bioforschung Austria, 2009). When no nodules are developed or nodules are inactive, nodulation failed, and soybean plants are deficient in nitrogen leading to a reduction in protein content. In this case, fertilization might be necessary to achieve an acceptable yield (Recknagel and Messmer, 2015).

In organic farming, weed is controlled mechanically. Therefore it is advised to plant soybeans in rows with a pneumatic sowing machine with a defined seed-to-seed distance, which enables weed management in the rows by finger weeder (Klaiss et al., 2020). In contrast, herbicides are employed in conventional farming for weed control, but this can unintentionally harm soybeans (Jürgen Recknagel, 2010) besides other negative environmental effects. Therefore, conventional farmers also often implement hoeing techniques. As a positive trait, soybeans do not require fungal treatment (Klaiss et al., 2020). Due to the relatively low cultivation density of soybeans, the incidence of pests and diseases is (still) low. The most significant fungal diseases in areas with high cultivation density are *Sclerotinia* and *Diaporthe/Phomopsis*, and the only pest potentially causing economic harm is the infestation with *Vanessa cardui* (Bernet et al., 2016). Furthermore, soybean production can be accomplished with the common machinery used by arable farmers (Höner, 2015), making it easier for farmers to begin producing (organic) soybeans.

1.4 Challenges of low temperatures during early plant development

Plant growth is primarily affected by temperature. Soybean is a thermophilic crop with an ideal temperature of 30°C and shows reduced or no growth below 10°C (Bachteler, 2016). The sowing date of soybeans is determined by the prevailing moisture and temperature conditions during spring. To minimize the risks of reduced yield due to immature stocks in autumn, soybeans are sown as early as possible, usually in late April or early May (Klaiss et al., 2020). To promote healthy plant development and reduce weed growth, farmers are recommended to sow soybeans at temperatures above 10°C (Klaiss et al., 2020). Temperatures below 10°C after sowing, can lead to reduced yields (Szczerba et al., 2021), as germination and emergence are delayed (Borowski and Michałek, 2014). The extent of yield depression depends on the soybean variety (Littlejohns and Tanner, 1976). Low temperatures decrease the final germination (Bharati et al., 1983; Posmyk et al., 2001; Mohammadi, 2009) and have detrimental effects on nodulation (Matthews and Hayes, 1982). Furthermore, decreased temperatures during the flowering stage can reduce the number of pods (Ohnishi et al., 2010) and subsequently lead to reduced yields (Staniak et al., 2021). The severity depends on the duration of exposure to these cold conditions (Kurosaki et al., 2003).

Good seed germination is crucial for successful seedling emergence. Germination (BBCH 05) refers to the stage where the radicle develops and is visible, while emergence (BBCH 09) is the stage where the young seedling reaches out of the soil. In laboratory experiments the use of paper or sand as medium is common and allows to report on germination. However, experiments conducted in pots or fields, usually report on emergence.

Delayed emergence increases seed susceptibility to diseases and pests such as Rhizoctonia and bean fly (*Delia platura*) (Bernet et al., 2016), whilst bird damage – particularly by pigeons (Schulz et al., 2024) – can result in complete loss (Höner, 2015). Bird attacks affect most likely young plants directly after emergence (BBCH 09 to BBCH 11) (Figure 2) (Gerbaulet, 2014).

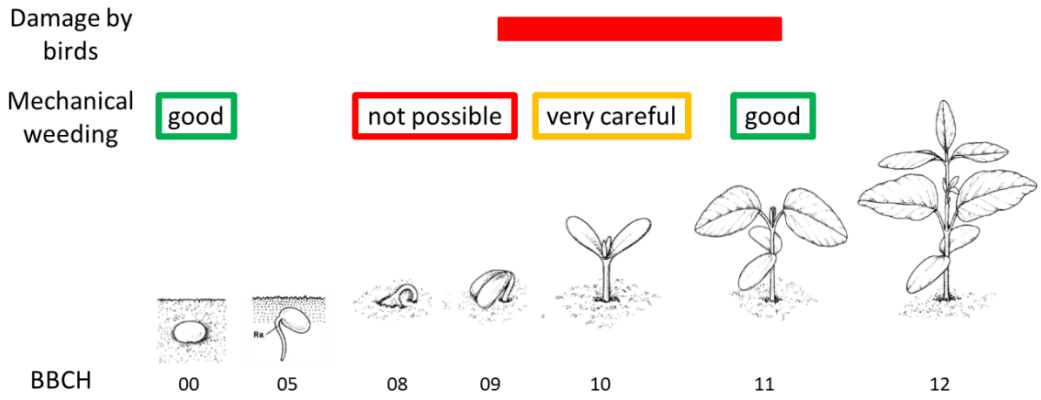

Figure 2 Early plant development, threats by birds and mechanical weeding possibilities. Own figure after Gerbaulet (2014) and Mücke (2016). Plant pictures taken from Meier (2018).

A patchy soybean crop stand is prone to be overrun by weeds, resulting in reduced yields (Schmidt and Langanky, 2019). Before germination or at the BBCH 11 stage, mechanical weeding is a viable option (Mücke, 2016). Once the plants reach BBCH 11, better BBCH 12, they overcome most of the obstacles encountered during their juvenile growth. Corbineau and Côme (2006) stressed the importance of high-quality seeds for successful crop establishment. Such seeds should have fast and uniform germination, with minimal sensitivity to external factors, allowing germination in a wide range of climatic conditions, even under harsh conditions (Corbineau and Côme, 2006).

1.5 Seed priming

Seed priming is the pre-sowing seed technique which enables faster germination and emergence. Germination consists of three phases: imbibition with rapid water uptake (I), a lag phase in which water uptake slows down and metabolic changes take place (II), and a final phase with further water uptake and radicle protrusion (III). Priming aims to permit a controlled water uptake by the seed until the end of phase II and stop the process before the radicle emerges from the seed since the seeds become very sensitive after that (Bradford, 1986). The metabolic changes in the seed entail various processes, including the activation of DNA repair and antioxidant mechanisms (Paparella et al., 2015). Therefore, priming enables the seed to undergo these changes during phases I and II, which are highly sensitive the external stresses such as drought (Corbineau and Côme, 2006).

Priming has been shown to enhance early plant development (Heydecker and Coolbear, 1977; Paparella et al., 2015), especially under environmental stress conditions, including drought (Langeroodi and Noora, 2017; Chatterjee et al., 2018), salinity (Ashraf and Foolad, 2005) and temperatures above (Corbineau and Côme, 2006) or below the optimal level (Bradford, 1986; Hardegree, 1996). Priming is widely used and adjusted for different crop species, such as vegetables and sugar beet, and various companies hold patented protocols (Paparella et al., 2015).

1.5.1 Types of priming

The priming techniques differ mainly in the solution used for seed imbibition. The water uptake is controlled by different means: Osmopriming and halopriming control the osmotic potential by the use of organic or inorganic ingredients, while the amount of water available is controlled when using wet material (solid matrix priming) or water vapour (Ashraf and Foolad, 2005). Hydropriming utilises pure water, and the duration of priming is crucial to control water uptake and prevent radicle emergence. This technique serves as the simplest option in terms of required materials and is described as a low-cost and low-risk method that enhances germination and emergence in various crops and can be applied on-farm (Harris et al., 1999). However, it is also used for commercial seed production, for instance, in crops like sugar beet and vegetables (Kockelmann et al., 2010; Paparella et al., 2015).

1.5.2 Priming in soybean

In various studies, the effect of priming on soybean germination, emergence, and yield was investigated with sometimes contradicting results. Priming methods in soybeans show beneficial effects on time to 50% emergence, total germination and yield (Arif et al., 2008; Mohammadi, 2009). Hydropriming of soybeans resulted in higher final germination at optimal growing conditions 25°C (Sadeghi et al., 2011), under lower temperatures, as they are found under German growing conditions

the results are inconsistent. Bharati et al. (1983) observed a decrease in emergence time at 10°C, while Mohammadi (2009) reported enhanced germination at 15°C. Zurheide et al. (2012) stated that faster germination occurred on paper, but lower final germination was observed in pot experiments. Decreased germination at 15°C was also reported by Ghassemi-Golezani et al. (2011).

Most studies use varying (hydro-)priming durations between a few hours and up to one day. A linear decrease in the mean germination time of soybeans by 2.4 hours for every hour of hydropriming was reported by Bharati *et al.* (1983). Moosavi et al. (2012) reported enhanced germination and yield in hydroprimed soybean seeds with an optimal priming duration of 8 hours. Over-priming soybeans, where seeds reach phase III of germination, can have negative effects and reduce germination, as also reported in other crops (Harris, 1996).

In most studies on priming in soybeans, the tests are conducted using a single soybean variety. Priming techniques probably show different responses among soybean varieties, due to varying seed sizes. Notably, Kering and Zhang (2015) demonstrated that priming accelerated emergence in larger seed varieties, while smaller and medium seed varieties exhibited less variation compared to non-primed control. Moshtaghi-Khavaran et al. (2014) identified a faster emergence of primed small seeds from the same variety and seed lot in comparison to larger seeds, which exhibited a lower response.

Hydropriming allows the seeds to absorb water, leading to an increase in size and weight proportional to the duration of hydration. Seed physical properties are crucial to know for adjusting agricultural machinery. Seed weight, expressed as thousand-kernel weight, is used to calculate the number of seeds to be sown per square metre. Any alterations in size and weight are of high importance for the practical application of hydropriming.

1.6 Seed additives

Seed additives describe the addition of any material or microorganisms to the seed before, during, or after sowing. Soil organisms have great potential to enhance sustainable ecosystem functioning, and soil ecological intensification can be an important step towards feeding the world while sustaining the planet (Bender et al., 2016). Biofertilizers comprise living microorganisms such as arbuscular mycorrhizal fungi (AMF), plant growth-promoting rhizobacteria (PGPR), and plant growth-promoting bacteria (PGPB) (Calvo et al., 2014). The positive impact on crop yield of these biofertilizers is widely reported (Schütz et al., 2018). Biological intensification is a fundamental principle in organic agriculture, which is gaining increasing popularity beyond the organic sector. In soils with low levels of biodiversity, the use of biofertilizers can result in increased species diversity and ecosystem functioning improvement (Bender et al., 2016). Conversely, in soils with high biodiversity, the impact is lower (Bender et al., 2016).

Inoculation with either PGPR or AMF results in improved nutrient supply to plants, achieved through symbiotic or free-living nitrogen fixing bacteria and an increased root surface facilitated by AMF (Adesemoye and Kloepper, 2009). Calvo et al. (2014) summarised the modes of action exhibited by biofertilizers as asymbiotic nitrogen fixation, nutrient solubilisation, siderophore production, production of volatile organic compounds, and synthesis of phytohormones. These mechanisms regulate physiological processes responsible for plant root development, cell division, senescence, germination, and growth. In a global meta-analysis, Schütz et al. (2018) found that legumes demonstrate the most positive yield effect through biofertilization, especially when the soil does not contain the corresponding nitrogen fixing bacteria, the addition of these led to yield increase.

1.6.1 Mycorrhizal fungi

Mycorrhizal fungi are soil fungi that can form a symbiosis with most terrestrial plants, including soybean (van der Heijden et al., 2015). The fungus increases the root surface area with its hyphal network, enabling the absorption of water and nutrients from a large soil volume (Nadeem et al., 2014). The fungus aids in the breakdown of nutrients into a more readily available form for the plant, in return the fungus receives sugars and fatty acids from the plant (Chen et al., 2018; Wipf et al., 2019). Mycorrhizae can assist in the solubilisation of phosphate, enhancing its availability to plants (Nadeem et al., 2014). The hyphal network additionally promotes soil aggregate stabilization and carbon sequestration, mitigating issues of erosion and leaching while simultaneously elevating water holding capacity (Chen et al., 2018).

Arbuscular mycorrhizal fungi (AMF) enter the root epidermis, with hyphae that run parallel to the endodermis inside the root cortex and form arbuscules inside cortical cells (Wipf et al., 2019). These

arbuscules are tree-like structures that are involved in nutrient exchange (van der Heijden et al., 2015). Host specificity is not common among mycorrhizal fungi, allowing the same fungus to colonise different plant species and connect them to a network of plants (Van Der Heijden and Horton, 2009). The widespread mycorrhizal networks enable inter-plant communication which can be employed in pest management among others (Wipf et al., 2019).

The AMF community composition in the soil is influenced by land use history, soil texture, pH, and plant density (Faggioli et al., 2019). According to Faggioli et al. (2019), soybean cultivation promotes a relatively high diversity of AMF. Adding other microbes to the soil can also change the local AMF community, for instance, AMF community diversity is increased when additional *Bradyrhizobia* is added to the soil (Omirou et al., 2016). In terms of AMF colonization, natural fungi occurrence is 30-60% greater in low-input systems (organic) compared to conventional systems (Mäder et al., 2000). High levels of plant-available P and Ammonia (NH^{4+}) negatively impact AMF symbiosis (Van Der Heijden and Horton, 2009; Wipf et al., 2019).

The addition of AMF products results in an average 20% increase in crop output worldwide (Schütz et al., 2018). Soybean production benefits when supplemented with AMF, leading to elevated nitrogen and phosphorous contents and yields of the crops (Schmidt et al., 2015). Schmidt et al. (2015) reviewed numerous pot experiments and indicated that AMF also improves rhizobial nodulation and nitrogen fixation, with numbers ranging from 25% to 118% more nodules and 12 to 320% more nodule weight. In addition to improved nodulation, better phosphorus nutrition is also reported upon AMF addition (Asimi et al., 1980; Mortimer et al., 2008). Asimi et al. (1980) found that the nodules' nitrogenase activity is stimulated, while Mortimer et al. (2008) reported an improvement in N2-fixation.

A large-scale field trial carried out by farmers using a commercial AMF product under conventional agricultural practices revealed a significant increase in potato yields following the application of AMF and the economic assessment revealed good profitability of the measure (Hijri, 2016).

The utilization of AMF in low-temperature environments has been shown to enhance biomass production (Chen et al., 2013), potentially compensating for the negative effects of cold temperatures (Chen et al., 2014). In a study by Zhang et al. (1995), a combination of mycorrhiza and nitrogen-fixing bacteria was tested on soybean at various low root zone temperatures (15, 18, and 21.6 degrees Celsius). The study revealed that the combination resulted in a decrease in the number of nodules, which was counterbalanced by an augmented mass per nodule, thus giving rise to an enhancement in N2 fixation. Several other studies have documented better plant growth under cold growing conditions with AMF (Chen et al., 2013, 2014; Schmidt et al., 2015). No studies were found focusing on the early plant development of soybean using AMF.

1.6.2 Plant growth-promoting rhizobacteria

Plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting bacteria (PGPB) combine various types of bacteria that have a positive impact on plant growth. The best-known PGPR is *Bradyrhizobia*, which develops symbiotic relationships with legumes and plays a crucial role in nutrient supply in organic agriculture. As *Bradyrhizobia japonicum* is not naturally occurring in Europe, seeds must be inoculated before sowing (Klaiss et al., 2020). There are multiple products and strains available for seed inoculation with *Bradyrhizobia japonicum*, with varying efficacy (Wächter et al., 2013; Zimmer et al., 2016). In addition to the commonly referenced *Bradyrhizobia japonicum*, other species have been found to form nodules in soybean roots, e.g., *B.elkanii*, *B.liaoningense, Mesorhizobium tianshanense, Sinorhizobium fredii and S.xinjiangense* (Rodríguez-Navarro et al., 2011). The success of nodulation also depends on the level of soil nitrogen available to the plant (Klaiss et al., 2020). Soybean nodulation is hindered by a high level of mineral nitrogen (Pan and Smith, 2000).

1.6.2.1 Development of nodules: genistein plays a crucial role

Soybean roots release signal molecules to communicate with rhizobacteria. One of these signal molecules is the isoflavonoid genistein, which triggers the expression of several bacterial nod genes. These genes, in turn, induce the synthesis of Nod factors by the bacteria, resulting in root infection (Kosslak et al., 1987). The initial phase of root infection involves the curling of root hairs, followed by the initiation and penetration of an infection thread (with the accompanying Rhizobia) into the root, then nodules grow through cell division and start to fix atmospheric nitrogen (N_2) to ammonia (NH_3) and ammonium (NH_4^+) (Zhang and Smith, 1994). Cold temperatures delay the infection process, while at 25°C the infection of roots takes three days, it is prolonged up to eight days under 15°C (Zhang and Smith, 1994). This effect can be counteracted by adding genistein to pretreat *Bradyrhizobia japonicum* before inoculation of soybean plants, which led to earlier onset of nodules under cold growing conditions (Zhang and Smith, 1997, 1995). Genistein addition leads to an increase in the number and weight of nodules (Belkheir et al., 2000; Pan and Smith, 2000; Dolatabadian et al., 2012), as well as higher shoot and root weight (Dolatabadian et al., 2013) and an increase in final nitrogen content (Belkheir et al., 2000). Additionally, under drought stress, the application of genistein led to the formation of more nodules (Nápoles et al., 2009). The addition of genistein can reduce the effects of salt stress (Miransari and Smith, 2007; Dolatabadian et al., 2012) and cold stress in soybean (Miransari and Smith, 2008). Two commercial products were tested in a large field trial in Canada and USA under low root zone temperatures, showing an average increase in grain yield of 7%, and even more (10%) in early planted soybeans (before the soil temperature reached 17.5°C) (Leibovitch et al., 2001).

1.6.2.2 Other PGPR

Several bacterial genera, including *Pseudomonas*, *Enterobacter*, *Bacillus*, *Variovorax*, *Klebsiella*, *Burkholderia*, *Azospirillum*, *Serratia* and *Azotobacter*, have also significant impact on plant growth (Nadeem et al., 2014). They have diverse modes of action that can be categorized as biofertilizers, phytostimulators, and biopesticides (Bhattacharyya and Jha, 2012). Biopesticides control phytopathogenic agents by producing substances that act against them or by inducing systemic resistance in plants. Phytostimulators are microorganisms that directly produce phytohormones, which affect plant growth (Bhattacharyya and Jha, 2012). Biofertilizers can be further classified as P solubilizers and N fixers (among which are symbiotic N fixers, such as *Bradyrhizobium japonicum)* (Schütz et al., 2018).

Plants treated with PGPR exhibit increased biomass in both roots and shoots (Kumar and Dube, 1992; Bai et al., 2003; Adesemoye and Kloepper, 2009; Kumar et al., 2016), as well as additional nodulation (Bai et al., 2003; Cassán et al., 2009; Masciarelli et al., 2014; Ferri et al., 2017). Reports regarding the effects of PGPRs on germination are scarce; however, Alahdadi et al., (2009) suggests improved germination. PGPR enhance plant nutrition through phosphate solubilisation and free-living nitrogen-fixing bacteria. They also produce phytohormones, such as auxins, cytokinins and ethylene, which play an important role in seed germination and can enhance host resilience (Schmidt et al., 2015). This leads to a higher nitrogen and phosphorus use efficiency, particularly in legumes (Schütz et al., 2018). Co-inoculation of *B. japonicum* with other PGPRs can improve soybean emergence and growth (Bai et al., 2003; Alahdadi et al., 2009).

1.6.3 Commercialization of biofertilizers

Numerous commercial products for biofertilization and thus improved plant growth are available. Chen et al. (2018) conducted a global market study and discovered approximately 75 companies producing AMF products in Europe, with Germany holding the largest market share, followed by Italy and Spain. To identify products worthy of further testing, market research was conducted between 2015 and 2017. Schmidt et al. (2015) previously provided an overview of available products containing PGPR and AMF in temperate regions. Further products were identified through literature (Zimmer et al., 2016) and web search.

Thirty-eight Products were found, and 27 of them are permitted for use in organic farming. Seventeen products have been identified for use in soybean cultivation. Of these, five contain *B. japonicum*, while three others remain unspecified about the active ingredient, but are also utilized for inoculation. Additionally, seven products containing PGPR, AMF, or other microbacteria were found to be designated for use on soybean or legumes. A further 19 products are not specified for soybean or

legumes (soybean was either not listed or target crops were not given). Most products contained a mixture of several microbial agents. *Bradyrhizobia japonicum* was determined and *Bacillus amyloliquefaciens* was identified as the predominant PGPR in the products (see Annex Table 22). Within the group of AMF, *Funneliformis mosseae* (previously known as *Glomus mosseae*) and *Rhizophagus irregularis* (previously known as *Glomus intraradices*) were the most frequently used.

2 Aim of the thesis

This study aimed to identify a methodological approach to accelerate and improve germination and early plant development of soybeans under temperate conditions, to shorten the period where plants are susceptible to pests and diseases and can be overgrown by weeds.

The study entails laboratory and pot experiments as well as one field experiment. To speed up the germination process, hydropriming was chosen as the underlying technique. Preliminary experiments were carried out to identify technical details, such as seed size after inoculation and the use of different types of water. Following, the effect of different durations of hydropriming on germination under cold temperatures (9 to 18°C) was studied.

To strengthen the early plant development of soybeans three seed additive products available to German farmers, i.e. one containing AMF, one PGPR and one containing genistein, were used in a pot experiment. In a field experiment on a German organic farm, the most promising hydropriming duration and one effective product were tested.

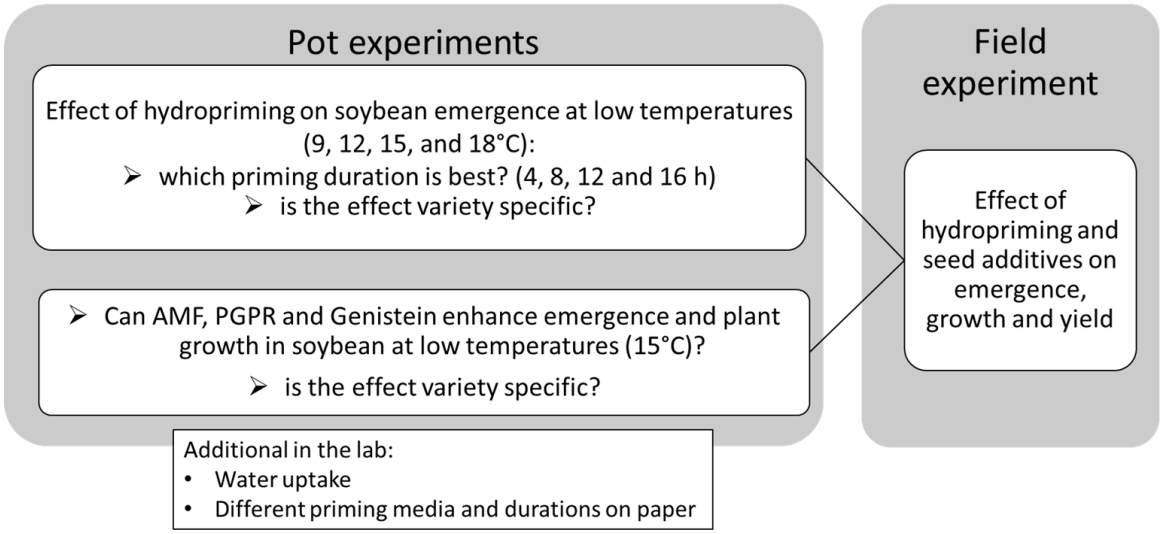

Figure 3 Overview of the research project

The main hypotheses of this study were:

(1) Hydropriming treatment leads to faster germination and emergence of soybeans and an increase in total germination and emergence as seen for other crop seeds.
(2) Germination is delayed with lower growing temperatures, but hydropriming can counteract this effect.
(3) The effects of hydropriming depend on the duration of priming.
(4) Using AMF, PGPR and genistein as seed additives improves emergence and early plant development.

(5) Soybean varieties differ in their response to hydropriming and seed additives due to their different sizes and maturity groups.
(6) Under field conditions, both hydropriming and seed additives show better emergence, crop establishment and yields.

All the materials and methods used are described in the next chapter, starting with the studies on hydropriming, followed by the screening of products and the field trial. The results are presented and discussed in the chapter Results and Discussion. The specific discussions are followed by a Synthesis of the research results, limitations and future perspectives of hydropriming and seed additives in soybean production.

3 Materials and Methods

In the following chapter, the materials and methods used over the whole study project are described. In the first part, the different studies concerning hydropriming are described, followed by the tests done with seed additives. The field experiment combining both is described, as well as further tests which were done concerning the seed quality during the study period.

3.1 Study on hydropriming effects

3.1.1 Seed material and hydropriming technique

In 2015 five different soybean varieties, broadly used in Germany (Table 1) were studied, to display a range of different seed sizes and maturity groups. Fresh seeds from the previous harvest (2014) were obtained from a seed company (Saatbau Linz eGen, Austria) except for the variety 'Primus' which was obtained from Gladbacherhof, the research station of Justus-Liebig-university Giessen in Villmar-Aumenau, Germany. The seeds were stored for ten months under cool (~ 4 to 8°C), dark, and dry conditions (30-52% relative humidity), except when transported (less than four hours).

Table 1. Description of soybean varieties used in the experiments. Thousand-grain weight and germination percentage according to the manufacturer information ('00': early, and '000': very early).

Variety	Available on the German market since	Maturity group	Thousand-grain weight (TGW) (g)	Germination percentage (%)
ES Mentor	2009	00	190	85
Lissabon	2008	000	184	91
Merlin	1997	000	190	91
Opaline	2009	00/000	203	94
Primus	2005	00	257	92

Unless otherwise specified, hydropriming was done in boxes (volume 600 ml) using distilled water and 100 soybean seeds. Water was not aerated and hydropriming was done at room temperature (~ 20 – 22°C) for four different durations (4, 8, 12, and 16 h). Non-treated seeds were used as a control (0 h). Seeds were dried on tablets with paper towels at room temperature (20 – 22°C) for 25 hours. The dry matter content (DM) of subsamples of seeds was determined (more than 48 h at 105°C) after 17.5 hours of hydropriming (ca. 40% DM) and after 25 hours drying at room temperature (ca. 85% DM).

3.1.2 Seed physical properties

Five seeds per variety were randomly located in a blister tray with 25 numbered places. One tray per hydropriming duration (4, 8, 12, or 16 h) was filled with distilled water and kept at room temperature

(22°C). Each seed was weighted and measured with an electric calliper before hydropriming, immediately after hydropriming and after 25 hours of re-drying in dry blister trays at room temperature. DM was determined after four days at 105°C. Weight and size changes through hydropriming were reported as percentages of the original weight and size.

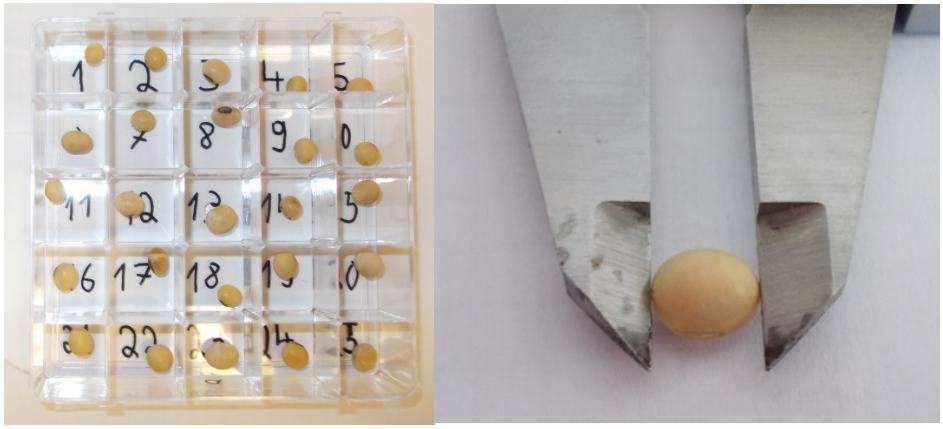

Figure 4 Random distribution of 5 varieties and 5 replicated seeds for the experiment on seed physical changes (weight and size). Here seeds were still dry. For each priming duration, one blister pack was used.

3.1.3 Emergence in pot experiment

The emergence of all five varieties after hydropriming treatment (0, 4, 8, 12, and 16 h) was tested in a pot experiment in climatic chambers at different temperatures (9, 12, 15, and 18°C) without light. A split-plot design was used with the chambers (i.e., temperatures) as blocks. In each chamber, four pots per combination (variety and hydropriming duration) were located completely randomized. Pots with 10 cm diameter were filled with a mix of one part loess soil and two parts quartz sand. Three seeds per pot (Figure 5) were placed 3 cm deep and the pots were watered up to 60% of the maximum water holding capacity, which was measured in advance and defined by the weight of the pot with substrate.

Figure 5 Pot experiment on hydropriming effect, left: seed placement in pots, right: emerged seed in pot (BBCH 09)

Every second day, the pots were watered again up to the defined weight. Emerged seeds (seeds reaching out of the soil surface, referring to BBCH 09) were counted daily until no further seeds emerged. In the 18°C chamber, the final count was done after 20 days, in the 15°C chamber after 21 days and in the 12°C chamber, the final count was done after 33 days. The seeds under 9°C showed only 11% of emergence and were therefore excluded from further analysis. The final germination was expressed as percentage. Mean time to emergence (MTE) was calculated as described by Ranal *et al.* (2009):

Equation 1 Mean time to emergence

$$\text{MTE} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$$

Where t_i is the time in days from the start of the experiment, n_i is the number of new seedlings emerged since the last observation and *k* the last time of observation.

3.1.4 Seed germination

Seed germination was tested with the variety 'Opaline'. Seeds (from the same seed lot as the other experiments, but stored for 1.5 years) were hydroprimed in plastic boxes with tap water (for parameters see Table 23) or distilled water for 4, 8, 12, and 16 hours each and untreated seeds as control. Afterwards, excessive water was removed with paper towels. The germination test was started immediately.

Figure 6 Germination tray with pleated paper and 50 soybean seeds, here is a pre-test, where not all hilums were placed upside.

In addition, seeds from hydropriming in distilled water were dried on tablets with paper towels for 25 hours at room temperature (~20-22°C) before starting the germination test. This resulted in four treatment levels concerning the hydropriming method, besides the duration: untreated seeds, hydropriming in tap water and direct sowing, hydropriming in distilled water and direct sowing,

hydropriming in distilled water and drying. The weight of seeds before and after hydropriming was measured and changes in weights were calculated as described above.

To test the germination, 50 seeds of each treatment were placed with the hilum upwards in boxes with pleated paper and moistened with distilled water (Figure 6). Each treatment was replicated four times. Boxes were randomly placed in a dark climatic chamber at 15°C. Germination (when the cotyledon became visible above the folds of the pleated paper, corresponding to BBCH 05) was counted daily for up to 28 days. Total germination is given in percentage and mean germination time (MGT) was calculated using the same formula described for MTE (Equation 1). Further germination characteristics were calculated as described by Ranal *et al*. (2009), such as germination rate ($\bar{v}$), uncertainty (*U*, an adaptation of the Shannon index), and synchronization index (*Z*).

3.1.5 Statistical analysis

Data was processed in SPSS Version 25 (IBM Corporation, 2017) using a full-factorial ANOVA with $p \leq 0.05$. Graphs were prepared with SPSS Version 28 (IBM, Corporation, 2021). Mean values per pot were used for statistical analysis, while single-plant data were used in graphs and some tables when indicated. Dependencies of hydropriming duration and size increase as well as hydropriming duration and weight increase were analysed with Pearson's r (2-tailed test).

Normal distribution of the residuals was checked by the Kolmogorov-Smirnov test for sample size > 50 and the Shapiro-Wilk test for sample size < 50. If data was not normally distributed but group sizes were equal, ANOVA was used as well, as in this case, it is robust to violations of normality (Field et al., 2012).

Homogeneity of variance was verified with Levene's test and according to Warton and Hui (2011), a violation of the equal variance assumption reduces the power to detect differences, leading to a more conservative result.

When differences were obtained, the Tukey HSD test was used for post hoc comparisons when variances were homogeneous and the Tamhane's T2 test when variances were heterogeneous; in both cases, a significance threshold of $p \leq 0.05$ was used.

3.2 Screening of different seed additive products

A fully randomised pot experiment was conducted for 55 days, starting in August 2015 in a climate chamber at the phytotron in Rauischholzhausen. The temperature was set at 15°C (the data logger measured an average temperature of 14.4°C, ranging between 13.6-15.8°C), at a relative humidity of 76%. No light was used for the first 14 days, and a 12-hour cycle was used from day 15. Pots of 10 cm diameter were filled with the same substrate as used in the hydropriming experiments (mixture of 1/3 loess soil and 2/3 quartz sand). One seed per pot was placed at a depth of 3 cm and the pots were watered to 60% of the maximum water holding capacity before the start of the experiment and every second day during the experiment.

Figure 7 Picture of the pot experiment on seed additives in the climatic chamber and while watering the pots

3.2.1 Seeds and Seed Additives

In this experiment, the same five soybean varieties from the same seed lot were used as in the previous pot experiment, for details see section 3.1.1 and Table 1. All seeds (except the control treatment) were inoculated with *Bradyrhizobium japonicum* using Biodoz® (DeSangose) since it was the most effective product which was available at this time (Zimmer et al., 2016). 4 g of Biodoz® was dissolved in distilled water (7.5 g) and 50 g of seeds of each variety were inoculated for a few minutes with 1.5 g of this mixture and directly planted.

Mykoplant® 100 BT-H (manufacturer Mykolife GmbH) contains AMF, namely *Glomus intraradices*, *Glomus etunicatum* and *Glomus mosseae*. The substrate consists of expanded clay granules, 10 g were applied to the seeding hole before the seeds were added to the pot. RhizoVital® 42fl. (manufacturer ABiTEP GmbH) contains different strains of *Bacillus amyloliquefaciens*. Following the manufacturer's description, the liquid was diluted (0.4 ml in 1 l of distilled water) and 1.5 ml of the solution was applied to the pots on top of the seeds using a multiple pipette. Genistein Soy Complex, is a powder made from soybean germ and advertised as being high in genistein (4 mg g^{-1}), daidzein (17 mg g^{-1}) and glycitein (10 mg g^{-1}), which is used for medicinal purposes. One teaspoon, equivalent to 0.08 mg genistein and 0.34 mg daidzein, was added to the soil before the seeds were placed in the pot.

Table 2 Shortcuts of the seed additives and their combinations used in the pot experiment

Abbreviation	treatment
C	control (no inoculation)
B	Biodoz® (inoculated control)
G	Genistein (with inoculation with Biodoz®)
GM	Genistein and Mykoplant® (with inoculation with Biodoz®)
GMR	Genistein + Mykoplant®+ RhizoVital® (with inoculation with Biodoz®)
GR	Genistein + RhizoVital® (with inoculation with Biodoz®)
M	Mykoplant® (with inoculation with Biodoz®)
MR	Mykoplant®+ RhizoVital® (with inoculation with Biodoz®)
R	RhizoVital® (with inoculation with Biodoz®)

Figure 8 Picture of the different tested products for seed additives: Mykoplant®, genistein and RhizoVital®

3.2.2 Data collection

For the first 30 days, the BBCH stages of the plants were recorded daily, followed by a record of when the plants were watered (Monday, Wednesday, and Friday). When the plants reached BBCH 12, the following parameters were measured

- Number of emerged seeds
- Plant height (from substrate to highest leaf)
- Number of leaves (open leaves including cotyledons)
- Chlorophyll content using a Yara handheld N-tester: 30 measurements per plant, to obtain the instrument's robust average in Yara units (dimensionless)
- Number of nodules (separate for main and side roots, but in the following the total number of nodules is used)
- Diameter of nodules on the main root
- Shoot and root fresh and dry matter

3.2.3 Additional pot experiment with RhizoVital® and genistein

To further investigate the effects of RhizoVital® and genistein, a second pot experiment was conducted in November 2016 using the variety 'Merlin'. It is the most referenced variety in soybean variety tests. The above-explained treatments 'B', 'G', 'R' and 'G+R' were tested by using a fully randomised design with 12 replicates. The experiment was conducted in a climate chamber at the Interdisciplinary Research Centre for Biosystems, Land Use and Nutrition (IFZ) at the Justus-Liebig-University Giessen. The temperature was set at 15°C with a day/night regime of 15 hours dark and 9 hours light and a constant humidity of 60%. Similar pots (10 cm diameter) and the same substrate (1/3 soil and 2/3 quartz sand) were used as before, with one seed per pot and watering to 60% water holding capacity. The position of the pots was randomly changed during the daily control and when watering was necessary.

Seeds were inoculated for all treatments. Due to availability, HiStick® Soy (BASF, 2015) was used, which is as effective as Biodoz®(Zimmer et al., 2016). All seeds were inoculated, therefore a small amount of HiStick® (4 g) was dissolved in distilled water (8 g), 24 seeds per treatment were inoculated with 0.4 g of the mixture. As in the previous experiment, RhizoVital®42fl. (manufactured by ABiTEP GmbH) was used, but at a higher concentration (1 ml RhizoVital® in 0.5 l distilled water). The seeds receiving RhizoVital® were rinsed with the RhizoVital® solution and drained before inoculation. Seeds treated with genistein 0.6 g of the same product as in the previous experiment were added to 2 g of HiStick® mixture and seeds were inoculated with this mixture.

BBCH of the plants was recorded daily and water was checked and refilled if necessary. After six weeks (when plants reached BBCH 12), plant height, number and size of nodules and fresh and dry matter of roots and shoots were measured using the same measurements as above.

3.2.4 Statistical analysis

Data analysis was done with R (version 4.2.2) and R studio (2022.12.0), and descriptive data were done in Excel. The germination data has a binary structure (0 for no plant, 1 for a plant); therefore, a binary test (Kruskal-Wallis) was chosen. The data for BBCH (and all other data collected) showed unbalanced data (as some plants did not grow), so the Scheffe test (using the R package "agricolae" (Felipe de Mendiburu, 2021)) was used as a post-hoc test.

To compare the two experiments on RhizoVital®, only data from 'Merlin' with the same treatments were taken from the first experiment and analysed with ANOVA using a two-factorial design with factors treatment and site.

3.3 Field experiment

3.3.1 Research site and experimental design

A field experiment was conducted in 2016 and 2017 at the certified organic research farm Gladbacherhof on the northwestern slope of the Taunus, Hesse, Germany (50°23'51"N 8°15'19"E). The soils are Luvisols (silty loam) (190 – 230 masl), the mean annual precipitation is 648 mm and the mean temperature is 9.5°C. The preceding crops were winter rye in 2016 and winter rye plus yellow mustard (catch crop) in 2017. The soil was ploughed and seedbed preparation was carried out three times, the last time on the day of sowing (for more details see Table 27).

Three soybean varieties were under study ('Merlin', 'ES Mentor' and 'Primus') and were combined with the treatment hydropriming for 12 h, the addition of Mykoplant® and the combination of both, and no treatment as a control. Each plot measured 4.5 m² (1.5 m x 3 m), with four rows spaced 35 cm apart and a seed-to-seed distance of 4 cm, with seeds placed in furrows by hand at a depth of 4 cm. A completely randomised block design with four replications was used, leading to 48 plots (see Annex for the site plans, Figure 33 and Figure 34). Additionally, the marginal plots outside of the randomized site were mechanically sown with soybean seeds ('Merlin') at a depth of 4 cm. In 2017, four of the eight marginal plots were sown with primed seed to see if this would damage the seed.

Seeds were sown on the 6th and 11th of May in 2016 and 2017 respectively. During the ten days after sowing, the mean temperature was 14.6°C with 13 mm of rainfall in 2016 and 15.5°C with 40.5 mm of rainfall in 2017 (Figure 10). Climatic data and soil temperature data (at 5 cm depth) were obtained from the experimental farm weather station, with comparable soils and similar orientations. The trial was covered with nets (to prevent damage by birds and rabbits) and weeded by hand and machine (depending on the need and feasibility of machine weeding). No fertiliser was applied.

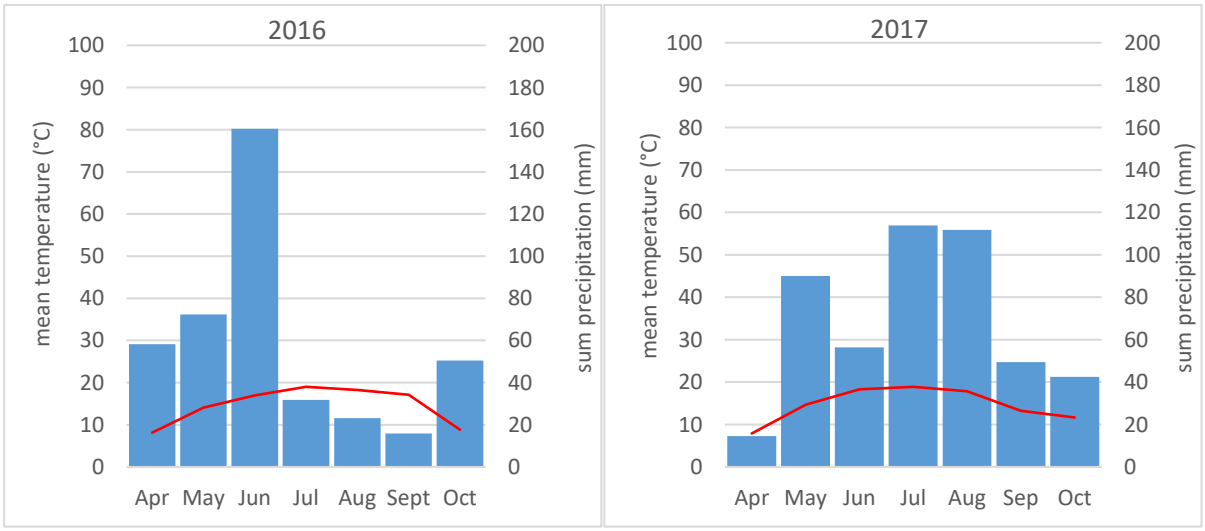

Figure 9 Climate chart for the growing period in 2016 and 2017. Mean temperature: red line, precipitation: blue bars.

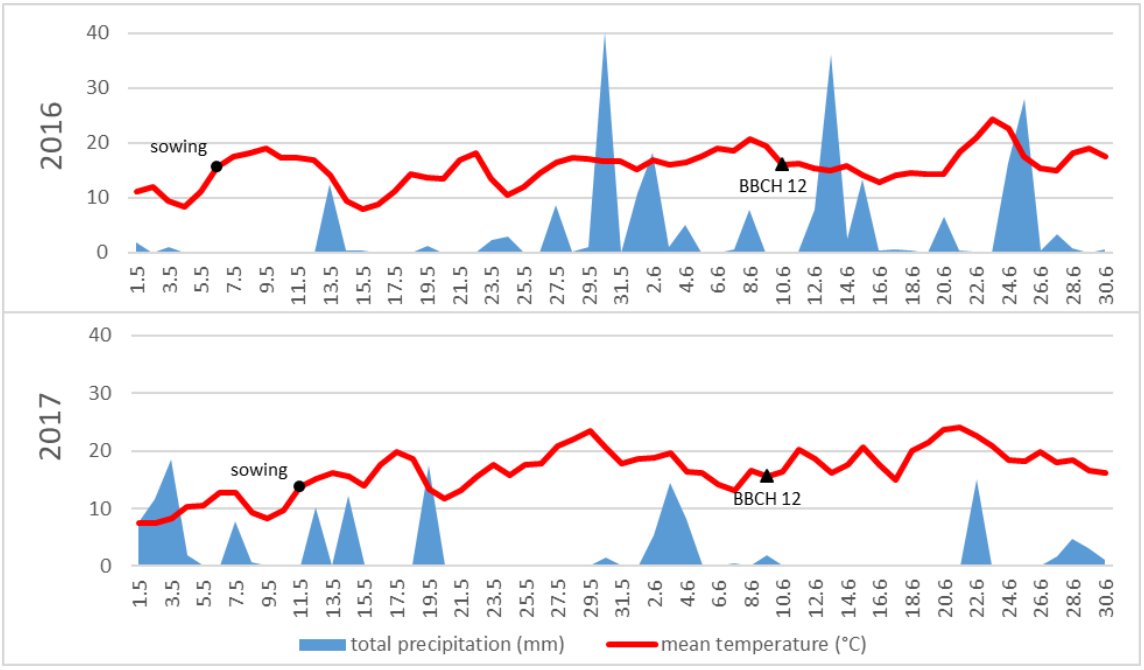

Figure 10 Mean temperature and total precipitation in May and June for the field experiment

3.3.2 Plant material

Three soybean varieties were selected to cover a wide range of seed sizes ('Primus' with the biggest seeds, followed by 'ES Mentor' and 'Merlin'), maturity groups ('Primus' and 'ES Mentor' '00', 'Merlin' '000') and two purposes, food ('Primus') and feed ('ES Mentor', 'Merlin') (Table 3). The soybean seeds came from different sources. Fresh seeds from the last harvest were used and stored in the dark at approximately 8°C and 50% humidity.

Table 3 Description of the plant material used in the field trial. The germination capacity shows the data that were given by the manufacturer or analysed at the research station.

Variety	**on the German market since**	**Maturity group**	**Seed size**	**2016** source	germ. capacity (%)	**2017** source	germ. capacity (%)
ES Mentor	2009	00	medium	Saatbau Linz	88	Saatbau Linz	91
Merlin	1997	000	small	Gladbacher-hof	98	Gladbacher-hof	92
Primus	2005	00	large	Gladbacher-hof	100	Taifun	87

3.3.3 Treatments

Hydropriming: Seeds were placed in tanks filled with tap water for 12 hours at room temperature (~ 20 - 22°C) without aeration. The seeds were then placed on tablets with paper towels to dry for approximately 25 hours at room temperature (ca. 20 - 22°C). Hydropriming was conducted before seeds were inoculated with *Bradyrhizobium japonicum.*

Seed inoculation: All seeds were inoculated with *Bradyrhizobium japonicum* using NPPL Force 48, which contains HiStick® Soy and a special glue (BASF, 2015). The inoculant was prepared as described in the instructions, using 1/3 HiStick® and 2/3 glue, and seeds were inoculated just before sowing. Seeds received more inoculant as described in the manual to ensure good inoculation, and if the time between inoculation and sowing was more than two hours, inoculation was repeated.

AMF Product: Mykoplant® 100 BT-H (Mykolife GmbH, 2016) was used, containing *Glomus intraradices*, *Glomus etunicatum* and *Glomus mosseae* on expanded clay granules with a natural nutrient component containing 2.4 mg/l nitrate, 1.2 mg/l phosphate, 2.6 mg/l potassium, 0.17 mg/l ammonium as well as some other micronutrients (see Table 26). Each seed in the plot received ½ teaspoon (approximately 0.5 g).

3.3.4 Data collection

Soil samples were taken before sowing and analysed for mineral nitrogen content (N_{min}). Between sowing and BBCH 12, plots were inspected daily to record their developmental stages (BBCH notation) (Meier, 2018), and weekly after BBCH 12. MTE in the field describes the days when most plants in the plot reached the stage of emergence (BBCH 09). At BBCH 12, several plant characteristics were measured from plants or areas in the centre of each plot (see Table 4): For germination, plants per metre of centre row were counted and expressed as percentages. Plant height was measured with a yardstick on 10 plants and the mean value was used. Chlorophyll was determined using a Yara N-tester (Yara GmbH, 2009) on 30 randomly selected plants. Leaf area index (LAI) was determined using an SS1 SunScan Canopy Analysis System (Delta-T Devices Ltd, Cambridge, UK) and two measurements were taken to give a mean value per plot.

Belowground parameters were taken from four plants at the edge of the plot (to avoid disturbing the centre of the plot), which were completely dug out to catch all the roots belonging to that plant. The roots of the plants were placed in buckets with sieves in tap water to soften the remaining soil. The remaining soil was manually washed off by moving the roots in the water and by hand. The fresh and dry weight of the shoots and roots and the dry weight of the nodules were taken. The shoot was cut, weighed and dried (at least 48 h at 105°C until constant weight), and then the dry matter was taken.

Nodules were removed from the root by hand or with tweezers and counted. Roots and nodules were weighed separately and dried in the same way as the shoot.

The height of the lowest (first) pod above the soil at 10 random plants per plot was measured just before the harvest with a yardstick. Harvest was done by hand at the appropriate date for each soybean variety (Table 4). Two frames (1 m x 0.75 m) were placed in the middle of the plot and all plants within the frames were cut near to ground. The plant material was transported in mortar boxes to the farm and total fresh weight was taken. Ten plants of each plot were selected randomly to count the pods per plant.

The harvested material was threshed using a laboratory thresher. Fresh seeds were weighed, and the weight of 1000 seeds was determined, and a sub-sample was dried (105°C, 48 h) to obtain the dry matter content. The yield is given based on a theoretical dry matter content of 86% (corresponding to 14% residual moisture, which is common in practice) and based on dt ha^{-1} (Equation 2). Other authors also make this moisture adjustment for yield data (Cafaro La Menza et al., 2017; Ferri et al., 2017).

Equation 2 Calculation of yield

$$Yield\ (dt/ha)\ with\ 14\%\ residual\ moisture = Yield\ DM\ (g/m^2) * \frac{100}{86} * \frac{10{,}000\ m^2\ per\ ha}{100{,}000\ g\ per\ dt}$$

A sub-sample of seeds was dried at 40°C for 12 hours and milled. Total N content was determined by Dumas combustion using the Elementar Analyzer Vario EL (Elementar Analysensysteme GmbH, Germany) with an analytical precision of 0.01 g N kg^{-1}. The dry matter of the milled beans was determined (drying at 105°C for 48 h) and the N content of the dry matter was calculated. Protein content was determined by multiplying the N content by 6.25 (see also Zimmer et al. (2016)) and also corrected for 86% DM. Data are expressed as percentages.

Table 4 Dates of harvests in the field trial, in dependency on soybean variety

Variety	**2016**	**2017**
ES Mentor	11.October	4. October
Merlin	14. September	27. September
Primus	29. September	18. October

Table 5 Overview of data collection within the field trial. Exact dates are given for both years. If no date is given, the data were not collected in the respective year.

Time	Traits taken	Date 2016	Date 2017
Before sowing	N_{min} (0-30 cm, one mixed sample per plot)	04 May	11 May
Between sowing and BBCH 12	BBCH stages checked daily	06 May to 10 June	11 May to 9 June
After BBCH 12	BBCH stages checked weekly	after 10 June until harvest	after 09 June until harvest
BBCH 12	Germination	10 June	9 June
	Crop height	10 June	9 June
	Chlorophyll	10 June	9 June
	Leaf area index	10 June	
	Nodules amount	10 June	7 to 9 June
	Shoot and root weight		7 to 9 June
	Nodules weight		7 to 9 June
Blossom	Crop height	14 July	11 July
	Chlorophyll	14 July	11 July
	Leaf area index (LAI)	14 July	
	Nodules amount	19 to 20 July	11 to 13 July
	Shoot and root weight		11 to 13 July
	Nodules weight		11 to 13 July
6 weeks after blossom	Crop height	19 August	
	Chlorophyll	19 August	
	Leaf area index	19 August	
Harvest	crop height	14 September to 11 October (see Table 4)	27 September to 18 October (see Table 4)
	height of first pods		
	pods per plant		
	Yield and biomass on 1,5 m²		
	Thousand-grain weight		
	N content (%)		
	Dry matter of beans and straw		

3.3.5 Statistical analysis

Data were processed in SPSS version 25 (IBM Corporation, 2017) using full factorial ANOVA with $p \leq 0.05$. Data were not transformed as this did not improve the homogeneity of variance or normality of residuals but would have made the data more difficult to interpret. The homogeneity of variance was tested using Levene's test. Some data showed unequal variances, but according to Warton and Hui (2011), violating the assumption of equal variances reduces the power to detect differences, leading to a more conservative result.

The normal distribution of the unstandardized residuals was tested using the Kolmogorov-Smirnov test when the sample size was > 50 and the Shapiro-Wilk test when the sample size was < 50. If the data were not normally distributed or had an ordinal structure, the non-parametric Kruskal-Wallis test was used and pairwise comparisons without p-correction (e.g. Bonferroni) were checked.

Following the ANOVA, post-hoc tests were performed as follows: a) if no significant interaction was found: for variables with normal distribution and equal variances, the Tukey test was used; if the variances were not equal, Tamhane's T2 test was used; b) if a significant interaction was found: the data set was separated by variety, then a post-hoc test for treatment effect for each variety was performed.

Correlations were analysed by Spearman correlation (as some data did not show a normal distribution and days are not interval scaled). Correlation values with $p > 0.05$ and $r_s < 0.5$ (or close to it) are considered as no correlation between the data.

3.4 Seed quality tests

All the tests compared in this chapter used soybean seeds (Table 1) that originated from the same seed lots from the 2014 harvest, as explained in more detail in 3.1.1. They were stored in cool (~ 4 to 8°C), dark and dry conditions (30-52% relative humidity). From July onwards, the seeds were exposed several times to room temperature or ambient conditions due to transport and handling. To get to know the quality of the seeds, four different pre-tests were performed.

3.4.1 Soak test

Taifun, a soybean processing company with its research centre, recommends the Soak test as a good and quick way to check seed quality, especially for seed coat damage (LZ soja Life Food, 2014). According to their protocol, seeds were soaked in water in a vessel and after 10 minutes, seeds were checked for blown seeds, which are assumed to have damaged seed coats. The soak test was carried out on 8 July 2015, using three replicates of 50 seeds each for each soybean variety. Blown seeds were counted and expressed as a percentage.

Figure 11 seeds after soak test. top left are blown up seeds. Picture taken from "Taifun-Sojainfo-Einweichtest für Soja-Saatgut", Author: Fabian von Beesten, 2014

3.4.2 Germination tests

In July 2015 (9 months of storage under controlled conditions), seed germination was tested: 25 seeds per soybean variety were placed in a petri dish (diameter 90 mm) between two layers of paper discs, using four replications per soybean variety. 15 ml of distilled water was added, and the petri dishes were stored at room temperature (~22°C). The germinated seeds were counted after seven days.

In November 2015 (13 months of storage), the germination test was repeated in germination boxes (50 seeds each) using pleated paper. The seeds were washed with tap water and placed between the folds with the navel upwards. 45 ml of distilled water was added to each tray, and they were incubated in a climate chamber at 25°C in the dark. After 8 days, final germination and the number of seeds with fungal infection were counted. Four replicates were made for each of the five soybean varieties.

In February/March 2016 (16/17 months of storage), the germination test on pleated paper (same method as in November 2015) was repeated with the variety `Opaline', but in a climate chamber at 15°C to study the effect of hydropriming (see also chapter 3.1.4). Each replicate contained 50 seeds and four replicates were used. Germinated seeds were counted after 28 days. For the data in this chapter, only data from the control group (not primed) were used.

3.4.3 Emergence tests

The pot experiments with hydropriming in July 2015 (9 months of storage) are described in detail in Chapter 3.1.3. All five soybean varieties were tested in pots at different temperatures: 9, 12, 15 and 18°C using a mixture of 2/3 sand from Rauischholzhausen and 1/3 field soil from Gladbacherhof as substrate. The emerging seeds were counted daily. For this chapter, only the control group (not primed) within each temperature was analysed. To calculate the mean emergence, the data of individual seeds (not the mean of the pots) was used.

In August 2015, the pot experiment to test seed additives was conducted, as described in Chapter 3.2. The same soil/sand mixture and pots were used as above, the temperature was set at 15°C, and all five soybean varieties were tested. Each pot contained one seed; emergence was recorded daily, and a final count was made after 30 days. For seed quality, analysis only the data from the untreated control and the treatment that had just received the *Bradyrhizobia* inoculation was taken.

Another pot experiment was set up in November 2016, for details see Chapter 3.2.3. One seed of the variety ‘Merlin’ per pot was used, with 12 replications and at a temperature of 15°C in growth chambers with light. Emergence was recorded daily, and the final count was conducted after 42 days.

3.4.4 LfL coldtest and germination test in an external laboratory

In January 2017, a seed sample of the variety 'Merlin' was sent to the Bayerische Landesanstalt für Landwirtschaft (LfL) to test germination and emergence using the so-called 'cold test': the seeds are placed on field soil under a temperature regime of seven days at 10°C followed by seven days at 25°C. They also carried out a germination test according to the ISTA standard (ISTA, 2015) (four replicates, 100 seeds each, 25°C final count after 8 days) using quartz sand.

3.4.5 Data analysis

The results were obtained from different experiments; therefore the set-up was not standardised. Means from different experiments or external sources were compared descriptively and by visualising differences and trends, no statistical analyses were performed.

4 Results and Discussion

4.1 Effects of hydropriming duration under cold growing conditions

4.1.1 Seed physical properties

The initial seed size varied between 7.3 mm ('ES Mentor') and 8.6 mm ('Primus'). 'Lissabon' had the lowest initial seed weight (186.8 mg) and 'Primus' was the highest (266.7 mg) (Table 6). This was in line with the variety classifications.

Table 6 Seed physical properties (weight and size) of the single varieties before priming.

	Initial weight (mg)	**Initial seed size (mm)**
ES Mentor	191.7	7.3
Lissabon	186.8	7.6
Merlin	190.9	7.9
Opaline	199.4	8.0
Primus	266.7	8.6

Hydropriming resulted in an increase in seed size from an initial 7.9 mm (mean for all soybean varieties) to 10.4 mm after 16 hours of hydropriming and subsequent drying. The average weight increased from 207.1 mg to 302.5 mg after 16 hours of hydropriming and drying.

Hydropriming duration and soybean variety had significant effects on weight and size gain and dry matter content (DM), while the interaction of soybean variety and hydropriming duration did not show significant effects on weight gain and DM responses (Table 7). The size increase was influenced by a significant interaction of variety and hydropriming (p=0.014) but with high variance. 'Merlin' and 'Lissabon' showed significantly lower size increases after 4 h of hydropriming compared to all other durations, while the other varieties did not differ significantly due to large variations between individual seeds (Table 9).

Table 7 F and p-values from ANOVA for the effects of soybean variety and hydro priming duration on weight and size of seeds.

source of variation	**df**	**weight gain after priming**		**weight gain after subsequent drying**		**size gain after priming**		**size gain after subsequent drying**	
		F	*p*-value	F	*p*-value	F	*p*-value	F	*p*-value
Variety	4	4.04	0.005	19.92	< 0.001	13.26	< 0.001	19.66	< 0.001
Priming duration	3	46.34	< 0.001	78.09	< 0.001	15.97	< 0.001	46.90	< 0.001
Priming duration-Variety	12	0.88	0.573	1.51	0.137	1.47	0.152	2.31	0.014

Table 8. Changes in seed physical characteristics after hydropriming and subsequent drying for 25 h. Values represent mean values and SD across all varieties. Values in the same column not sharing the same letter are significantly different at p < 0.05, tested with Tukey HSD

	Priming duration (h)	Seed weight increase (%)	DM (%)	Seed size increase (%)
Total	4	15.7 ± 7.2 a	77.8 ± 4.7 a	17.3 ± 6.4
	8	26.5 ± 6.9 b	70.9 ± 4.1 b	24.8 ± 5.4
	12	32.0 ± 9.4 c	67.7 ± 5.0 c	28.8 ± 8.0
	16	41.1 ± 8.9 d	63.3 ± 4.2 d	33.1 ± 6.6

Table 9 Seed size increase (%) depending on hydropriming duration and soybean variety. Values represent mean values and SD. Values in the same column not sharing the same letter are significantly different at p < 0.05, tested with Tamhane´s T2

Priming duration (h)	ES Mentor	Lissabon	Merlin	Opaline	Primus
4	22.3 ± 6.8 a	16.9 ± 2.1 a	11.6 ± 1.8 a	13.8 ± 5.1 a	21.9 ± 7.5 a
8	21.4 ± 2.2 a	24.5 ± 1.4 b	19.9 ± 1.4 b	29.1 ± 8.6 ab	29.0 ± 2.0 ab
12	31.0 ± 5.8 ab	28.1 ± 2.7 b	20.1 ± 7.4 abc	26.5 ± 4.7 b	38.4 ± 6.4 bc
16	39.1 ± 5.8 b	30.5 ± 3.2 b	25.4 ± 1.2 c	29.4 ± 3.8 b	39.5 ± 3.2 c

Positive correlations were found for hydropriming duration and size increase ($r = 0.655$, $p < 0.001$) and for hydropriming duration and weight increase ($r = 0.749$, $p < 0.001$). 12 hours of hydropriming resulted in a 32% increase in seed weight, a 29% increase in seed size and an average seed DM content of 68%.

4.1.2 Seed emergence after different priming durations

The mean time to emergence (MTE) in all pots was 14 days, ranging from seven days ('Merlin', 18°C, 16 h hydropriming) to 28 days (‘Lissabon’ and ‘Opaline’, 12°C, 4 h hydropriming). The MTE was 20.3 days at 12°C, 12.6 days at 15°C and 9.5 days at 18°C (Table 10). No significant interaction was found between soybean variety and hydropriming duration (Table 25). MTE was significantly dependent on the interaction between hydropriming duration and temperature (p=0.021) and between variety and temperature (p=0.005). At 15°C and 18°C, MTE did not differ for the duration of hydropriming. At 12°C, there was a significant decrease in MTE of 2.2 days from control (21.8 days) to 12 hours of hydropriming (19.6 days) (Table 11). All other hydropriming durations showed no significant differences. In some variety and growing temperature combinations (e.g. ‘Primus’ at 15°C and ‘Lissabon’ at 12°C) the plants showed less data spreading compared to control (Figure 12).

Table 10. Mean time to emergence (MTE) and total emergence of soybean seeds in response to temperature in climatic chambers in the pot experiment. Data show mean values and SD of single plant data across five varieties.

		MTE (days)	**Emergence (%)**
Total mean		14.02 ± 5.27	68 ± 47
Temperature (°C)	12	20.33 ± 3.32	66 ± 47
	15	12.56 ± 2.49	67 ± 47
	18	9.47 ± 1.98	71 ± 46
Hydropriming duration (h)	0	14.02 ± 5.53	79 ± 41
	4	14.07 ± 5.13	55 ± 50
	8	14.24 ± 5.66	69 ± 46
	12	13.63 ± 4.75	69 ± 46
	16	14.13 ± 5.24	68 ± 47

Table 11. Results of MTE depending on temperature and hydropriming duration and mean emergence depending on hydropriming duration in the pot experiment. MTE data and statistics based means of pots, which hold each three seeds). Values represent mean values and standard deviation (SD) across all soybean varieties. Values for MTE in the same column not sharing the same lower-case letter are significantly different at $p < 0.05$ tested with Tukey HSD. Values in the same row (for MTE) not sharing the same upper-case letter are significantly different at $p < 0.05$, tested with Tukey HSD. Emergence tested with Median test.

	MTE (days)			Emergence (%)
Hydropriming duration (h)	12°C	15°C	18°C	mean
0	21.8 ± 2.9 a A	12.9 ± 2.0 a B	9.3 ± 1.3 a C	78.9 ± 28.8 a
4	20.0 ± 3.1 ab A	12.5 ± 2.2 a B	9.4 ± 1.0 a C	55.0 ± 30.0 b
8	21.2 ± 2.9 ab A	12.7 ± 1.8 a B	9.6 ± 1.5 a C	69.4 ± 28.3 ab
12	19.6 ± 2.1 b A	11.8 ± 1.8 a B	10.0 ± 1.6 a C	68.9 ± 30.6 ab
16	20.5 ± 2.5 ab A	13.0 ± 2.7 a B	9.7 ± 1.6 a C	67.2 ± 29.1 ab

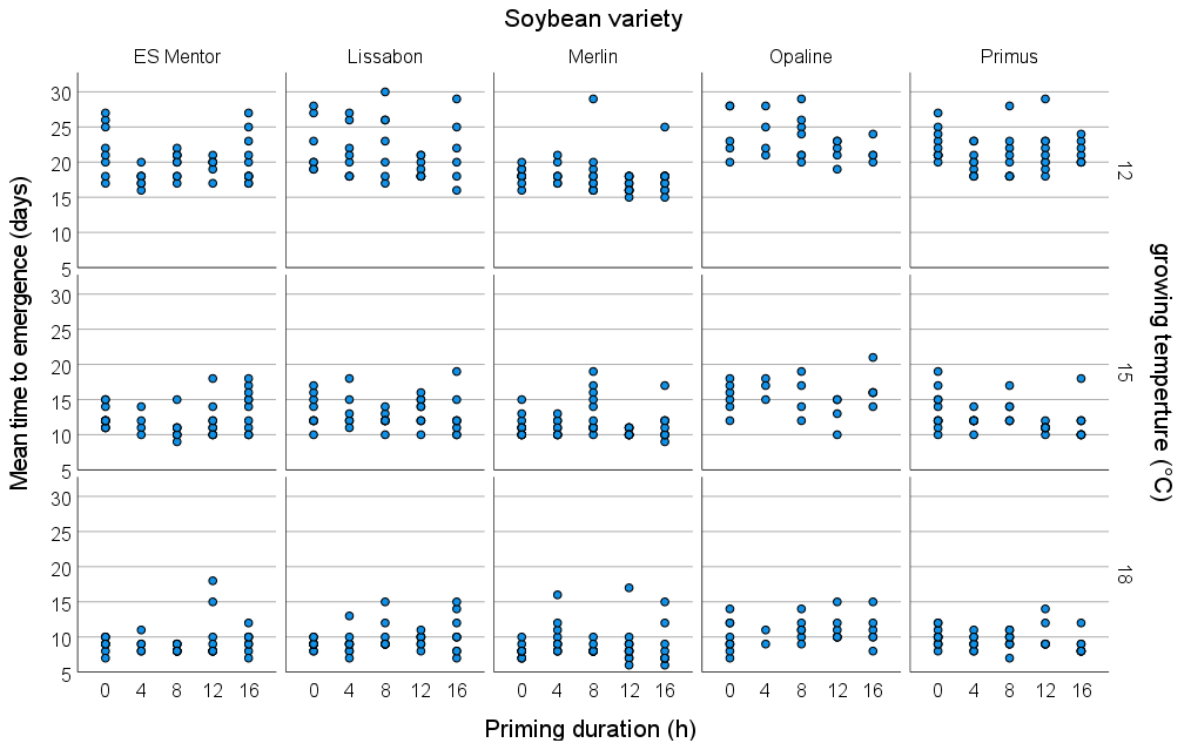

Figure 12. Mean time to emergence (MTE) in pot experiment depending on soybean variety, seed hydropriming duration and temperature in climatic chamber. Dots represent single plants.

Total emergence over all tested treatments in the pot experiment was 68%, ranging from 17% ('Opaline', 18°C, 4 h hydropriming) to 100% ('Merlin') (Figure 13). Neither temperature nor the temperature-priming interaction effects were significant (both $p > 0.05$). Variety ($p < 0.001$) as well as hydropriming duration ($p < 0.001$) affected seed emergence significantly. 'Opaline' showed a very low emergence with 43% overall. The highest emergence was observed in 'Merlin' (88%), followed by 'Primus' (71%) (Table 12). The highest emergence was found for the control (79%) and lowest after four hours of hydropriming (55%), with the other hydropriming durations varying between 67 and 69% emergence (Table 11).

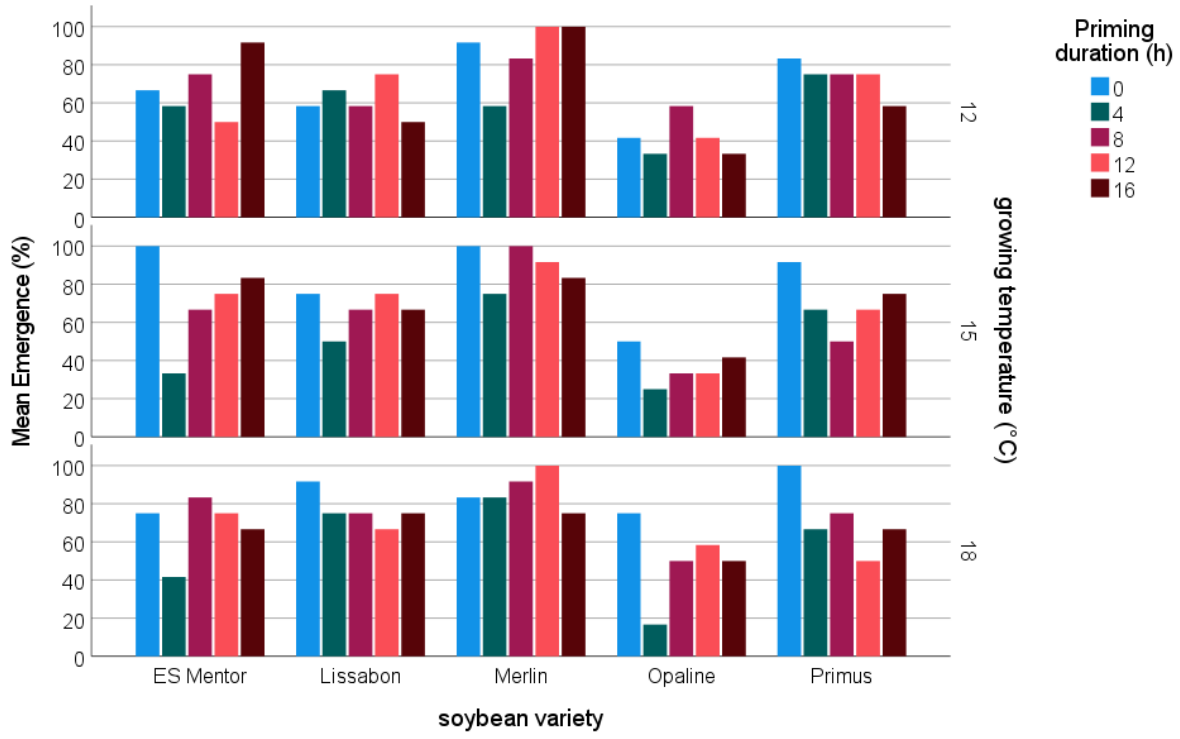

Figure 13 Total emergence in pot experiment depending on soybean variety, seed hydropriming duration and temperature in climatic chambers. Based on single plant data.

Table 12. Mean time of emergence (MTE) and percentage emergence depending on soybean variety in the pot experiment. Values represent mean values and SD across all hydropriming durations. Values in the same column not sharing the same lower-case letter are significantly different at $p < 0.05$, tested with Tukey HSD for MTE and Median test for Emergence. Values in the same row (for MTE) not sharing the same upper-case letter are significantly different at $p < 0.05$, tested with Tukey HSD.

		MTE (days)		Emergence (%)
Variety	12°C	15°C	18°C	mean
ES Mentor	20.0 ± 2.3 b A	12.1 ± 1.5 a B	9.1 ± 1.0 a C	69.4 ± 29.0 b
Lissabon	21.4 ± 3.3 bc A	12.9 ± 1.8 a B	9.6 ± 1.4 a C	68.3 ± 25.6 b
Merlin	17.9 ± 1.5 a A	11.3 ± 1.5 a B	8.7 ± 1.2 a C	87.8 ± 19.4 a
Opaline	23.2 ± 2.1 c A	15.0 ± 2.3 b B	10.8 ± 1.4 b C	42.8 ± 31.9 c
Primus	21.2 ± 1.7 bc A	12.4 ± 2.2 a B	9.6 ± 1.4 a C	71.1 ± 25.6 ab

4.1.3 Seed germination after replacing distilled water with tap water and without drying

Germination on paper was highest in the control (59.5%), and all tested hydropriming durations as well as all tested media led to a significant reduction of germination ($p < 0.001$). No significant differences in germination capacity were found for the different hydropriming durations, as well as between the different media.

Seed germination was around two days faster after 12 and 16 hours of hydropriming compared to the control without hydropriming (germination after 17 days) (Table 13). The type of water used for hydropriming (distilled water vs. tap water) did not influence total germination. Both media decreased

germination time compared to the control but using distilled water and subsequent drying of seeds for 25 hours did not lead to an improvement of germination time.

Germination rate ($\bar{v}$) was increased by hydropriming with distilled water or tap water without drying from 0.06 seeds per day to 0.07 seeds per day. No significant effect was found in the germination synchrony (*U* and *Z*, for more data, see Table 24).

Table 13. Germination (%) and mean germination time (MGT) (days) depending on hydropriming duration as well as medium in germination test on paper at 15°C. Values represent mean values and SD. Values in the same column (for the same parameter) not sharing the same subscript are significantly different at p < 0.05. Germination tested with Tukey HSD. MGT with Tamhane's.

		Germination (%)	**MGT (days)**
Hydropriming duration (h)	0	59.5 ± 8.3 a	17.1 ± 1.3 a
	4	25.5 ± 7.0 b	15.9 ± 2.1 ab
	8	23.5 ± 6.3 b	15.7 ± 1.8 ab
	12	26.4 ± 7.0 b	15.2 ± 1.1 b
	16	25.8 ± 6.2 b	15.0 ± 1.7 b
Medium	Distilled water	25.6 ± 7.3 b	14.6 ± 1.1 b
	Distilled water. dried	25.6 ± 7.5 b	16.9 ± 1.7 a
	Tap water	24.5 ± 4.6 b	14.6 ± 1.1 b

4.1.4 Discussion on hydropriming effects on soybean seeds

4.1.4.1 Hydropriming speeds up germination and emergence only at 12°C

The hypothesis was that hydropriming would improve germination and emergence and that an optimal hydropriming duration could be determined. Contrary to the expectations based on other studies (e.g. Mohammadi (2009) and Prasad and Prasad (2010)), the emergence wasn't faster with primed seeds under cold growing conditions of 15 and 18°C. This is in line with findings from Costa et al. (2013) at 25°C and Ghassemi-Golezani (2011) at 15°C. At 12°C as well as in the germination test on paper, hydropriming of 12 hours decreased the time of emergence and germination by two days, therefore this duration can be considered the best under the coldest of the evaluated conditions. This is in line with a previous study showing faster emergence after 12 hours of hydropriming (Arif et al., 2008). In a meta-analysis of various Iranian studies, a priming duration of 12 - 24 hours showed the best changes in emergence rate and crop yield (Soltani and Soltani, 2015).

After 12 hours of hydropriming, a moisture content of 32% was measured. According to Corbineau and Côme (2006) the moisture content of primed seeds is maintained around 40% to avoid germination before sowing. This indicates that the duration of priming could be prolonged to reach 40% moisture

to further improve the germination, assuming it was done without detrimental impacts on the seed oxygen supply.

At 12°C, 'ES Mentor' and 'Lissabon' showed a much more synchronized emergence after 12 hours of hydropriming compared to the control without priming (Figure 12). At higher temperatures, however, the opposite effect was observed. Future research should therefore include a standardized parameter for the synchrony of germination, e.g. the Z-index that is described in an overview of measurements of the germination process and a description of the calculation of numerous germination parameters (Ranal et al., 2009).

Faster germination and emergence with increasing temperature were found, which is in line with other studies (Hopper et al., 1979; Bharati et al., 1983; Mohammadi, 2009; Borowski and Michałek, 2014). Additionally, no differences between distilled water and tap water were found, indicating that the type of water doesn´t make a difference.

4.1.4.2 Hydropriming lowers total germination and emergence success

In all experiments, total germination was decreased after hydropriming, which is in contrast to many studies that describe an improvement in seed germination after hydropriming treatments without aeration and with different protocols for drying seeds after priming (Bharati et al., 1983; Arif et al., 2008; Moosavi et al., 2012; Arif et al., 2014; Langeroodi and Noora, 2017). This discrepancy is discussed in more detail in chapter 5.1 Synthesis. Other authors showed reduced total germination after hydropriming treatments using distilled water without aeration and four hours of priming followed by four hours of drying under room temperature (Helsel et al., 1986), or five and 10 hours of priming, without any information about drying (Kering and Zhang, 2015). Arif et al. (2008) reported declining total emergence with increasing hydropriming duration and showed better results for osmoprimed seeds.

4.1.4.3 Soybean varieties did not differ in their reaction to hydropriming treatments

The hypothesis was that soybean varieties differ in response to hydropriming treatment. Even though there were always significant effects of soybean varieties, there was no interaction between soybean variety and hydropriming duration. Lewandowska et al. (2020) similarly did not find a significant interaction between priming and soybean varieties under field conditions for most parameters at harvest.

The significant effect of soybean variety on total germination and time to emergence was not surprising, as seed germination percentages vary between the different varieties. Total germination was not affected by growing temperature, which is in line with findings from Bharati et al. (1983), but showed a large discrepancy when compared to the manufacturer's information, particularly in

‘Opaline’, which had only 43% germination. The mean time to emergence can be ranked as ‘Merlin’ (fastest) < ‘ES Mentor’ < ‘Primus’ < ‘Lissabon’ < ‘Opaline’ (slowest). ‘Opaline’ from the ‘00/000’ group and ‘Lissabon’, one variety in the earliest maturity group tested (‘000’) showed, contrary to the expectation, the slowest emergence. ‘Merlin’, the other variety of the earliest maturity group in the study, showed not only the fastest emergence but also the highest total emergence (88%). This variety is therefore often recommended for temperate growing conditions with a short vegetative period.

4.1.4.4 Seed size increased with increasing hydropriming duration

Seed size increase, as well as weight increase, correlated positively with hydropriming duration. After 12 hours of hydropriming (which was the most promising hydropriming duration found in the current studies) the size increased by 32%, which will require an adjustment of the sowing machinery. After 12 hours of hydropriming and 25 hours of drying the seeds had a DM content of 68%, which should be > 84% to be allowed for trading in Germany (for granulated or encrusted seed this does not apply) (Federal Office of Justice, 2006, p. 56). Seeds must be dried to a defined DM while maintaining the positive effect of priming. In the experiment on paper, faster germination after hydropriming was diminished when seeds were dried. The same was observed by Bharati et al. (1983), who found faster and more synchronized germination after hydropriming pre-treatment under cold laboratory conditions (10°C), but these effects disappeared with subsequent dehydration of seeds, including at different drying temperatures (47°C and 25°C) and final moisture contents of seeds (20% and 12%). Corbineau et al. (2006) also stressed the lack of consensus on drying and storage methods for the primed seeds. For practical purposes, it is important to dry the seeds after priming, to stop the process of germination, prevent mould infestation, make seeds storable, and reduce their susceptibility to damage while handling and sowing.

4.2 PGPR, AMF and genistein do not improve early plant growth

4.2.1 Variety-specific effects of different products

4.2.1.1 Germination

Treatment or the soybean variety had no significant effect on the final germination (Table 14). The mean germination in the experiment was 82%, and the highest values were obtained by 'Primus' and 'Merlin' (each 89%), while the highest final germination was observed under treatment genistein and Mykoplant® (93%) (Table 16). The plant development was significantly influenced by variety, and only the time to reach BBCH 10 showed a significant interaction between treatment and variety. 'Opaline' was the slowest variety in all developmental stages and took 51 days to reach BBCH 12. The addition of Mykoplant® showed the fastest emergence (BBCH 09), which was significantly higher than the combination of all three products (Table 16), but without showing significant differences in later plant development (stages BBCH 11 and 12) (Table 15).

Table 14 Statistical analysis of the final Germination data within the seed additive pot experiment.

	Germination	
test used	Kruskal- Wallis	
	Chi^2	p-value
variety	4.7282	0.3163
treatment	5.4324	0.7105

Table 15 F and p-values by ANOVA for the effect of variety and treatment with seed additives on plant developmental stages expressed as BBCH stages in the pot experiments on seed additives.

	BBCH 09		**BBCH 10**		**BBCH 11**		**BBCH 12**	
test	ANOVA		ANOVA		ANOVA		ANOVA	
	F value	p Value	F value	p Value	F value	p Value	F value	p Value
variety	8.2521	< 0.001	21.8655	< 0.001	7.1838	< 0.001	18.9199	< 0.001
treatment	4.4478	< 0.001	3.4521	0.0023	0.4193	0.9054	1.2008	0.3126
variety-treatment	1.7353	0.0423	2.8670	< 0.001	0.8887	0.6358	0.4379	0.9939

Table 16 Germination in dependence of soybean varieties and seed additive treatments. FG=final germination in % and time to reach the stages BBCH 09 to 12, given in days. Letters indicate significant differences within one developmental stage (Scheffe test used for post hoc comparisons).

		FG (%)	BBCH 09		BBCH 10		BBCH 11		BBCH 12	
variety	ES Mentor	78 a	11.80	b	17.71	c	27.43	b	46.86	b
	Lissabon	85 a	12.79	ab	19.30	b	28.87	ab	43.39	c
	Merlin	89 a	11.22	b	18.00	bc	28.04	b	45.96	bc
	Opaline	70 a	14.75	a	22.00	a	31.05	a	50.78	a
	Primus	89 a	12.00	b	18.25	bc	26.33	b	45.04	bc
treatment	control w/o inoculation	73 a	14.00	ab	19.64	ab	28.09	a	45.82	a
	Biodoz®	80 a	12.78	ab	19.67	ab	29.08	a	46.00	a
	Genistein	80 a	12.50	ab	18.73	ab	28.58	a	45.83	a
	Mykoplant®	87 a	10.64	b	17.69	b	27.85	a	45.62	a
	RhizoVital®	87 a	12.75	ab	18.08	ab	27.38	a	46.85	a
	Genistein + Mykoplant®	93 a	12.33	ab	19.29	ab	28.21	a	45.85	a
	Genistein + RhizoVital®	87 a	12.50	ab	18.77	ab	28.00	a	47.08	a
	Mykoplant® + RhizoVital®	87 a	11.17	b	18.54	ab	28.15	a	47.54	a
	Genistein + Mykoplant® + RhizoVital®	67 a	15.00	a	20.67	a	29.10	a	44.60	a

4.2.1.2 Plant traits at BBCH 12

No significant effect was found for the number of leaves (data not shown). All other above and below-ground plant parameters (plant height, chlorophyll content, shoot and root DM, number and size of nodules) showed a significant effect of soybean variety (Table 17). Chlorophyll content showed a significant treatment effect: the lowest value was found in the combination of all three products ('GMR') (Figure 14). The number and size of nodules were only significantly different in the non-inoculated control, where no nodules were found, indicating that soil was free from *Bradyrhizobia japonicum* (Figure 15). The combination of genistein and RhizoVital® showed a big range in the number of nodules, with a median higher than the control.

Table 17 ANOVA results of F and p-values by ANOVA for the effect of variety and treatment with seed additives on above-ground plant parameters at BBCH 12 in the pot experiments on seed additives.

	plant height		Chlorophyll		shoot DM	
	F value	p Value	F value	p Value	F value	p Value
variety	35.0105	< 0.001	29.2748	< 0.001	29.6324	< 0.001
treatment	0.4236	0.903	5.0214	< 0.001	1.5642	0.1527
variety-treatment	0.6657	0.896	1.7881	0.024	0.5565	0.9645

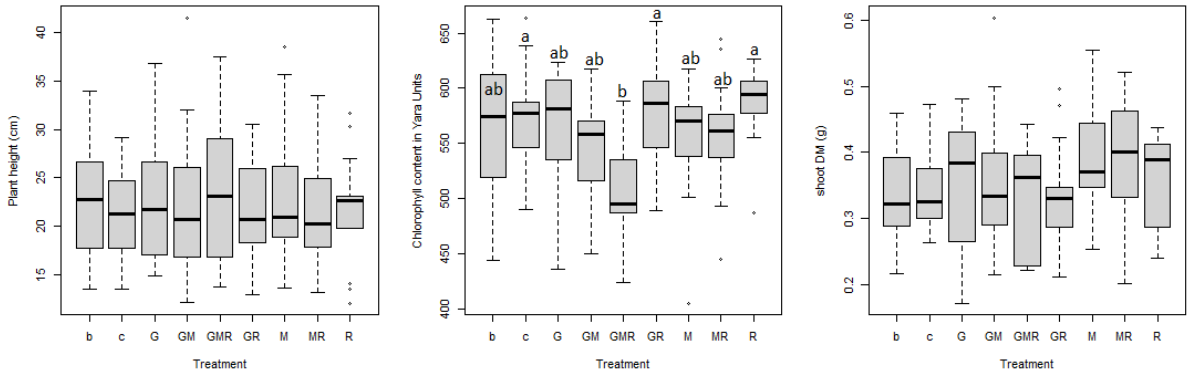

Figure 14 Above ground biomass grouped for treatment as boxplots. b= only Biodoz® (B.japonicum), c=control without inoculation, G=genistein, M=Mykoplant® (VAM), R=RhizoVital® (PGPR) in various combinations. Different letters show significant differences between the treatments.

Table 18 F and p-values by ANOVA for the effect of variety and treatment with seed additives on root parameters of plants at BBCH 12 in the pot experiment on seed additives.

	no of nodules		nodules size		root DM	
	F value	p Value	F value	p Value	F value	p Value
variety	14.7049	< 0.001	4.0574	0.0059	25.1230	< 0.001
treatment	10.8492	< 0.001	3.4252	0.0041	1.1268	0.3573
variety - treatment	1.7686	0.0257	1.0201	0.4612	0.9323	0.5764

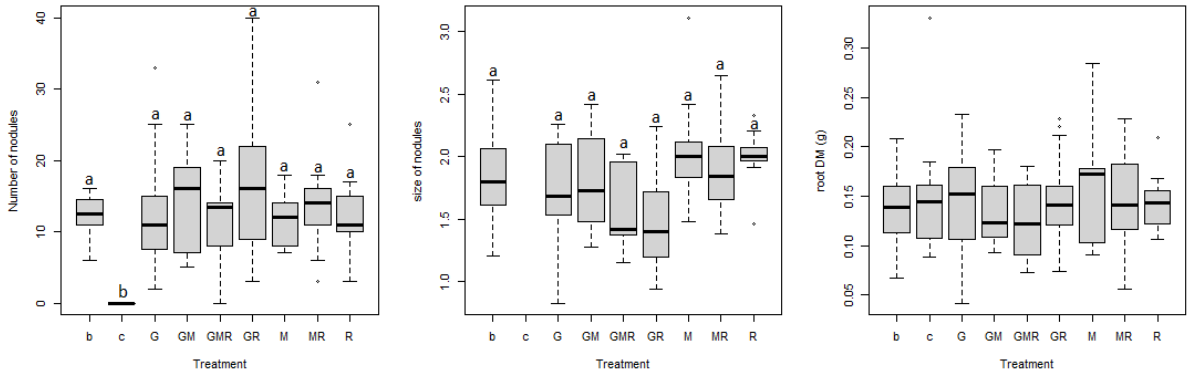

Figure 15 Below ground biomass: Boxplots for treatments with seed additives. The letters indicate the treatment as follows: b=only Biodoz® (B.japonicum), c= control without inoculation, G=genistein, M=Mykoplant® (VAM), R=RhizoVital® (PGPR) in various combinations. Different letters show significant differences between the treatments.

4.2.2 Additional pot experiment with RhizoVital® and genistein

Very few seeds emerged (23%), none of the RhizoVital® treated seeds emerged, only 33% of the twelve seeds treated with *Bradyrhizobia* and 33% of the one seed receiving additional genistein and 25% of genistein+RhizoVital® (Figure 16). No significant differences were detected in all studied traits.

The early plant development showed a mean duration to reach BBCH 09, 10 and 11 of 14.2, 19.5 and 23.2 days, respectively. This is comparable to the values found for 'Merlin' in the former study. A trend to faster development was shown by the addition of genistein (Figure 17). Root and shoot DM tended to be higher in genistein combined with RhizoVital® (Figure 18). Nodules and plant height did not show any differences between the treatments.

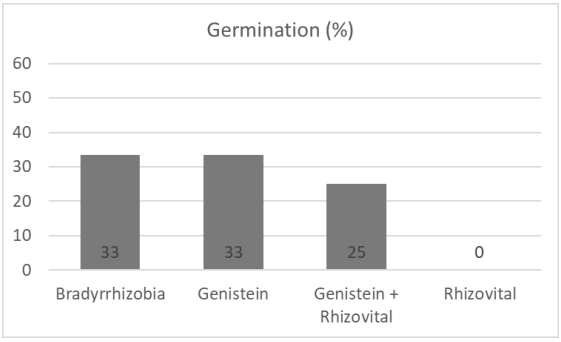

Figure 16 Germination of seeds in the additional pot experiment at IFZ on seed additives.

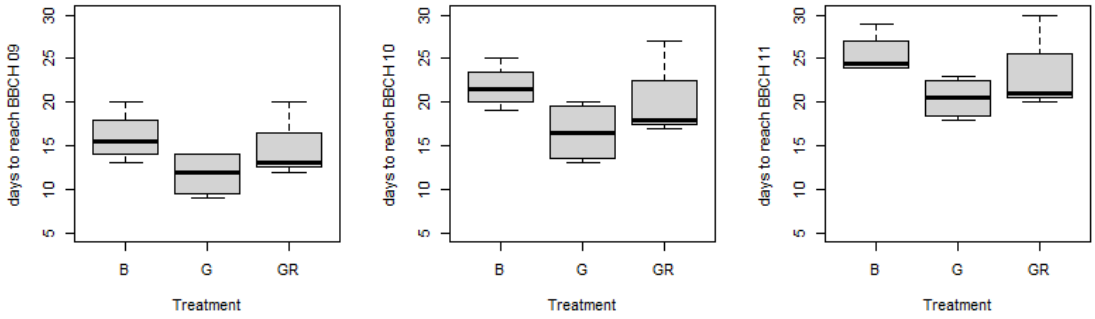

Figure 17 Days to reach BBCH stages in the additional pot trial at IFZ in dependence on seed additive. The letters indicate the treatment as follows: B=Biodoz® (B.japonicum), G=genistein.

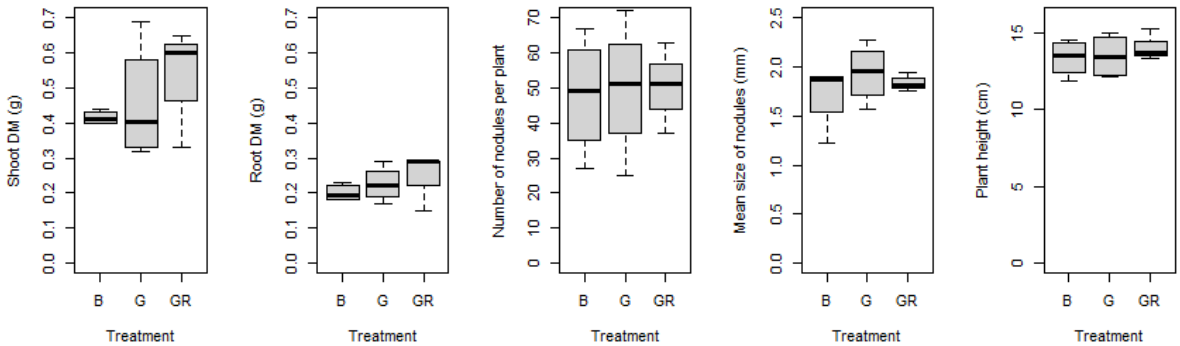

Figure 18 Further plant traits taken in the additional pot experiment at IFZ in dependence on seed additive. The letters indicate the treatment as follows: B=Biodoz® (B.japonicum), G=genistein.

4.2.3 Comparison of two studies (IFZ and RH)

Combining both datasets, again no significant effect of treatment was found. The plants in the experiment at IFZ were faster in BBCH 11, even if there were no significant differences in BBCH 9 and 10. In IFZ, not all plants reached the BBCH 12 stage, when the height was taken, while in RH all plants reached BBCH 12, this could be a reason for the difference in the plant height.

Table 19 Comparison of the mean values of different plant traits from the different experiments on seed additives (distinguished as sites)

site	BBC 09 (days)	BBCH10 (days)	BBCH11 (days)	plant height (cm)	nodules total (number)	nodules size (mm)	shoot DM (g)	root DM (g)
IFZ	14.2 a	19.5 a	23.2 a	13.6 a	49.3 a	1.83 a	0.460 a	0.221 a
RH	12.1 a	18.8 a	28.2 b	21.3 b	12.1 b	1.65 a	0.411 a	0.150 b

4.2.4 Discussion on Seed Additives

4.2.4.1 Comparison of the two sites

In the IFZ experiment, the plants showed more nodules and higher root DM. In the experiment at RH lower amount of *Bradyrhizobia* was used: nearly the same mixture, but 1.5 g of it for 50 g of seeds. Here 0.4 g of the mixture was used for 24 seeds. Thus, in the experiment approximately the triple amount of inoculation was applied, which could explain the higher number of nodules found at IFZ. Since the DM of roots also contains the nodules, this fact can also explain why the root DM was higher in IFZ.

4.2.4.2 No impact on nodule development

Since the soil used in the experiment originated from a field site, which was not cultivated with soybean before and *Bradyrhizobium japonicum* is not native to German soils, the control without any seed inoculation resulted in no nodules. Beside that, the seed additives did not show a significant effect towards the number and size of nodules within the co-inoculated seeds, this is contrary to the results of a meta-analysis, reporting increased nodule numbers after co-inoculation of *Bradyrhizobium* with *Bacillus* strains (Zeffa et al., 2020). The treatments with RhizoVital® (containing *Bacillus amyloliquefaciens*) and Mykoplant® (with different Glomus strains), and the combination of both showed a trend to bigger nodules, without changes in the number of nodules or chlorophyll content. This is contrary to Zhang et al. (1995), which found a decrease in nodule number which was counterbalanced by an increased mass per nodule, thus giving rise to an enhancement in N_2-fixation, when combining mycorrhiza and nitrogen-fixing bacteria at various low root zone temperatures (15, 18, and 21.6 °C). The findings of the current study regarding nodule development are also contrary to

Masciarelli et al. (2014), who found improved nodulation (more nodules on principal and secondary roots) when co-inoculate soybeans with *Bradyrhizobium japonicum* and Bacillus amyloliquefaciens. In the second experiment, which used more inoculation, more nodules were found, which is in line with former studies (Albareda et al., 2009; Hungria et al., 2017).

4.2.4.3 No improvement in emergence and plant development

Contrary to the initial hypothesis, all seed additives showed neither a significant effect on final germination nor on the speed of germination compared to the untreated control. Mykoplant® showed a faster emergence (BBCH 09) compared to the combination of all three products, but this effect was not found later in BBCH 11 and BBCH 12. No increased biomass production could be found, as plant height, shoot and root DM and number of leaves did not differ from control, and no sign differences between the treatments could be found. This is in contrast to other studies of AMF and *B. japonicum* which showed a positive influence on early plant development (Yadav et al., 2013; Prasad, 2021; Wang et al., 2011), as well as on emergence (Kloepper and Scher, 1987). The addition of the PGPR *Azospirillum brasilensense* to *B. japonicum* enhanced seed germination and early development of soybean seedlings (Cassán et al., 2009) and higher yields (Hungria et al., 2015).

In the second study plants treated with genistein showed a trend to emerge faster, but this could not be statistically proven, this is in line with former studies (Zhang and Smith, 1995, 1997).

4.2.4.4 Negative effect of the combination of all three products

The combination of PGPR and AMF did not show significant differences from the seeds, which just received Biodoz®. This is in contrast to other studies showing enhanced biomass and yield production of co-inoculation of *B. amyloliquefaciens* and AMF, but without additional *B. japonicum* inoculation (Sheteiwy et al., 2021). In other plants, the combination of *Glomus mosseae* and *Bacillus amyloliquefaciens* showed no improvement over the use of *Glomus mosseae* alone (Pan et al., 2020).

The combination of all three products showed the slowest emergence, lowest final germination and reduced chlorophyll content, while the combination of PGPR and AMF alone showed no reduction. The addition of genistein (which is expected to improve nodule development (Zhang and Smith, 1995)) seems to harm plant development. A temperature dependency of the effects of co-inoculation of *B. japonicum*, PGPR and genistein is reported with antagonistic effects under 15°C while showing positive effects at 25°C (Dashti et al., 2014). The soybean plants have to supply their symbiosis partner with carbon, which is why soybean plants co-inoculated with AMF and *Bradyrhizobia* show increased leaf area and C assimilation (Harris et al., 1985). The supply of three symbiotic partners may exceed the resources of the young plant.

4.2.4.5 'Variety' showed a stronger effect on plant development than seed additives

Variety-treatment interactions were only found in BBCH 09 and 10, in chlorophyll content and nodules number. However, due to the low number of plants within each treatment-variety combination, a further statistical analysis was not feasible. The inclusion of five varieties in the research design can be questioned. Research is always limited due to financial resources, time and/or space. In this case, it would have been better to have a lower number of varieties, but more replications. This would make the data analysis more robust. All traits (except final germination) are significantly influenced by soybean variety. This visualizes the importance of choosing and breeding suitable soybean varieties.

4.2.4.6 Limitations

Due to limited access to climatic chambers, the study used only three replicates per treatment soybean variety combination, and each replicate consisted of only one plant. Results are based on three plants per combination, which might be too low for obtaining robust research findings. The results are therefore more to read as trends. As the project time plan had to be kept, the best option for the field experiments had to be chosen based on the trend, which was found in the pot experiments. No product clearly showed positive effects, but as Mykoplant® showed some positive trends, it was chosen for the subsequent field experiment.

4.3 Field experiment to test hydropriming and AMF

Soil N ranged between 11 and 71 kg N_{min} ha^{-1}. This is within the range of the long-term mean for the pre-crop rye (followed by cereals) of 35 kg N_{min} ha^{-1} (Landesbetrieb Landwirtschaft Hessen, 2019). N_{min} concentration in soil before sowing differed significantly between the years; this might be due to the different sites on the farm. In 2016, the N_{min} was on average 25 kg N_{min} ha^{-1}, ranging from 11 to 43 kg N_{min} ha^{-1} within the plots. In 2017, the average was 50 kg N_{min} ha^{-1}, ranging from 32 to 71 kg N_{min} ha^{-1} in the plots. The soil N content was negatively correlated with the number of nodules and chlorophyll content at BBCH 12 and positively correlated to the crop height at blossom. However, no correlation was found for chlorophyll content at blossom or nodules at blossom.

4.3.1 Early plant development

The total germination ranged between 22 and 100%. The mean emergence is 70%, which is in line with other reports of field emergence (Voit, 2016). The treatments with hydropriming showed significantly lower germination compared to the control as well as to Mykoplant® alone (Figure 19).

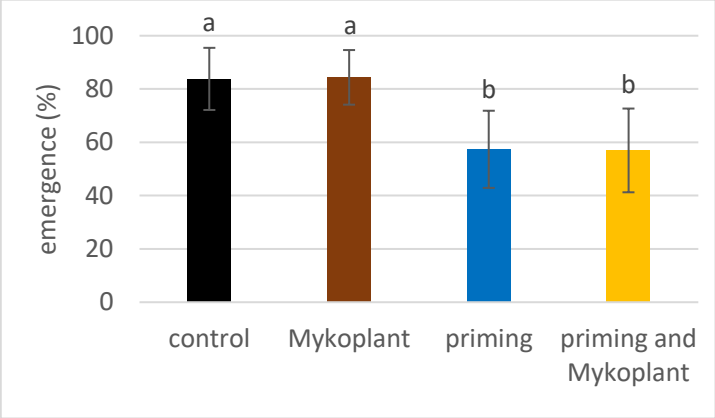

Figure 19 Effect of treatment on total emergence in field trail. Mean and SD over both years and three soybean varieties, different letters indicate significant differences.

On average BBCH 09 was reached after 14.75 days with a high variation of a minimum of eight days and a maximum of 22 days. BBCH 10 was reached after 17 days, BBCH 11 after 20 days and BBCH 12 after 29 days. No significant effect of the treatments was found (Figure 20). Significant effect was found for the years, due to different temperatures and rainfall conditions (for more details see *Figure 9*). After sowing in 2016 the temperatures were high, but it was quite dry, after seven days there was rainfall and lower temperature. The plants took 21 days after sowing to reach BBCH 09 in 2016. In 2017, the conditions after sowing were quite good (rainfall and rising temperatures), thus, BBCH 09 was reached after eight days. A negative correlation ($r = -0.8$, $p < 0.001$) between soil N content before sowing and the days to reach the different stages, thus a higher soil N content might have led to faster emergence.

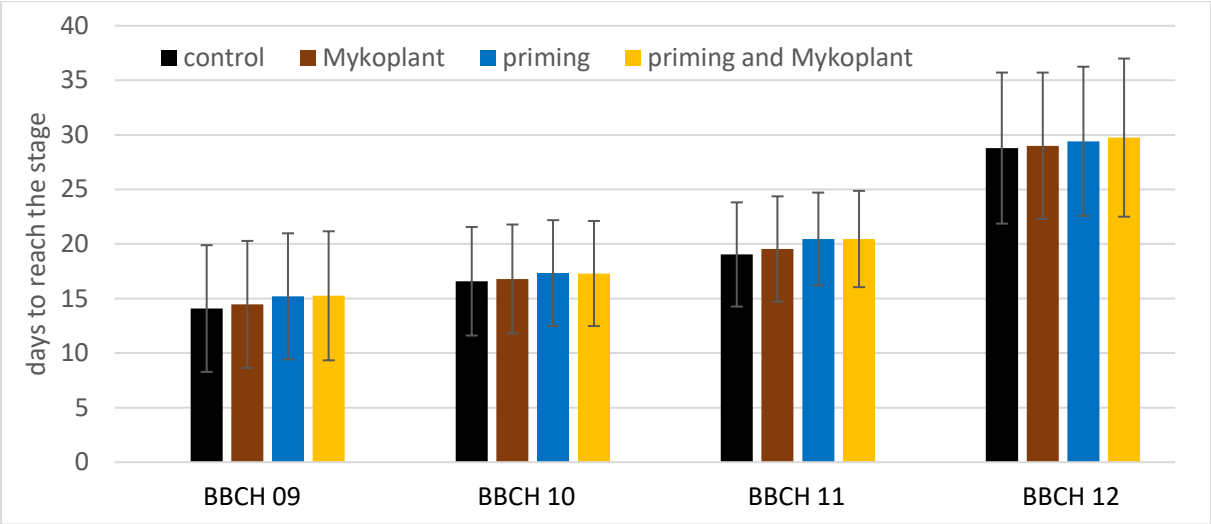

Figure 20 Early plant development in the field experiment, given as days to reach the BBCH stages 9, 10, 11 and 12. Mean values and SD over both years and three soybean varieties.

4.3.2 Root and nodules

Roots were not affected by priming or AMF treatment. The number of nodules at BBCH 12 was significantly increased after priming treatments.

4.3.3 Yield

The soybean yield varied between 15 to 70 dt ha^{-1}, with an average of 46 dt ha^{-1}. This is quite high compared with national variety trials, they reported a mean over all tested varieties of 39 dt/ha in 2016 and 40 dt/ha for 2017 (LLH, 2018, 2017), but in experimental field trials the management and harvest is optimized with a lot of manual work. Priming and well as priming+Mykoplant® led to a significant reduction in yield in the soybean variety 'Primus', the other varieties did not show significant differences (Table 28). No relevant correlation with other characteristics and yield could be found (see Annex, Table 30). The N content of the dry matter was on average 7.42% and showed no effect of treatment. N content and thousand grain weight (TGW) were positively correlated. The mean value of TGW was 188 g DM and the plants from primed seeds showed significantly lower TGW compared to control. A positive correlation between TGW and plant height at harvest was found.

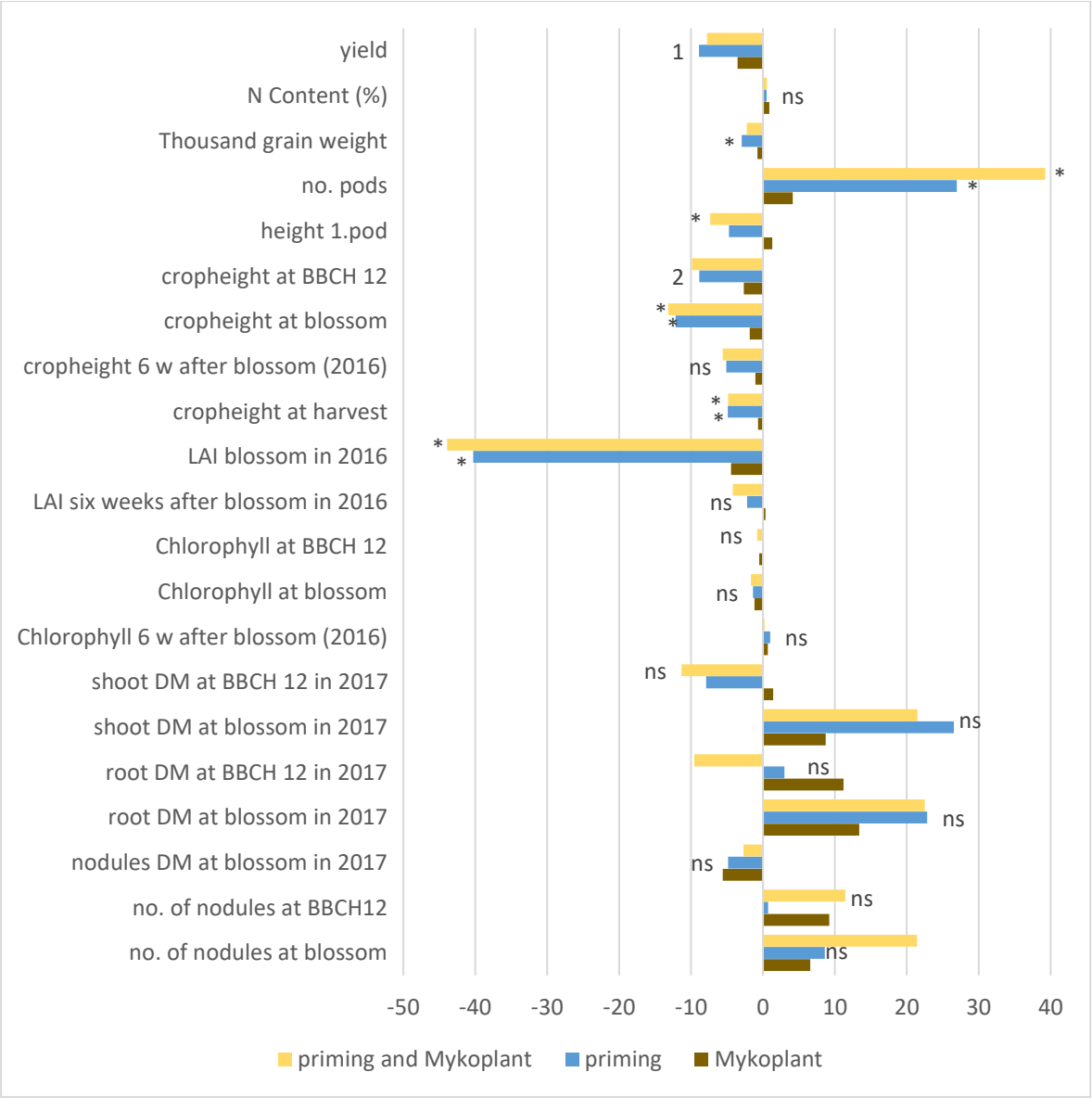

Figure 21 Percentual changes of the treatments compared to control for different parameters of the field experiment. Asterisks mark significant differences compared to control (*), test for significant differences were done by Tukey of the original data. ns: no significant effect. 1: within 'Primus', priming and priming in combination with Mykoplant® had significant lower values than the control. 2: within ES Mentor in 2017, priming and priming in combination with Mykoplant® were significant lower.

Number of pods per plant varied between 16 and 50, with a mean value of 29 pods per plant. Both treatments including priming led to a significantly higher number of pods per plant compared to control (Figure 22). The height of the first pod above the soil was on average 14 cm (ranging from nine to 20), priming in combination with Mykoplant® led to a reduced height of the first pods. Further, the height of the first pods was positively correlated to the crop height at blossom.

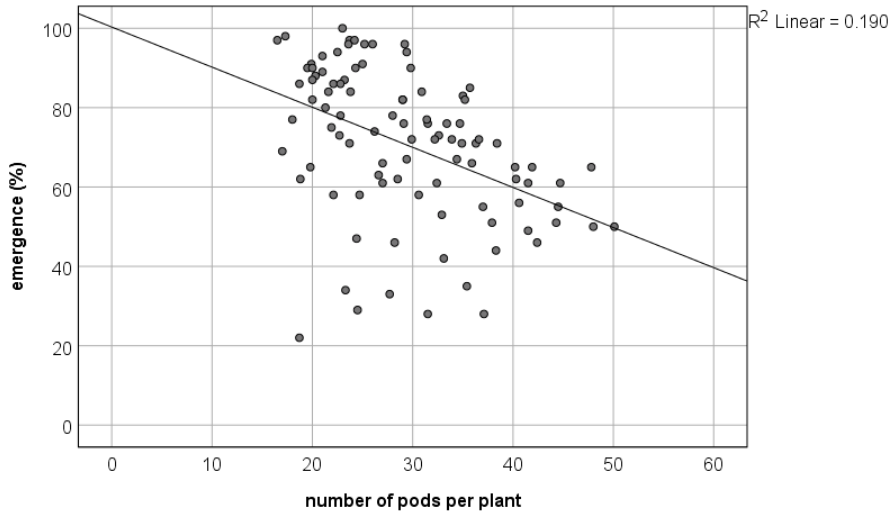

Figure 22 Correlation between number of pods and emergence in the field experiments. Values for both years and all three varieties.

4.3.4 Other parameters

Crop height at blossom and harvest showed significantly lower values for plants from primed seeds (Figure 21). The height at BBCH 12 and six weeks after harvest showed the same trend but without significance. Plants from primed seeds had significantly lower LAI at blossom, so more light reached the ground, but at blossom, this effect was reduced, and no differences were found. Chlorophyll content, shoot and root DM did not show differences at all, as well as the number and DM of nodules.

The way of sowing (hand vs. mechanical sowing) seems to have less effect on the germination of primed seeds. The hand-sown seeds (of the variety 'Merlin', which was also used in the marginal plots) showed 26% lower germination after priming compared to the control. The emergence in mechanical sowing was 24% lower in primed seed compared to control.

Table 20 Germination (%) of soybean variety 'Merlin' in the field experiment 2017. The data for hand sowing were part of the randomized field experiment, the drill seed data originated from the marginal plots.

sowing method	control	primed	% difference
hand	96.0 ± 2.2	71.3 ± 6.4	- 25.8
drill seed	77.0 ± 13.9	58.3 ± 13.3	- 24.4

4.3.5 Variety effect

Nearly all tested parameters (except root and shoot DM at BBCH 12) showed significant effects of soybean varieties (Table 28) which is not studied further. For this study, only significant interactions were of interest. These were found for emergence, height at BBCH 12 and yield.

For emergence ‘Primus’ showed a treatment-year interaction, in both years the treatments including priming were lower, in 2017 priming reduced the emergence by more than 50%. ‘ES Mentor’ and ‘Merlin’ showed significantly reduced emergence. No significant treatment effects on yield were found in ‘ES Mentor’ and ‘Merlin’, but ‘Primus’ showed significantly reduced yield when seeds were primed.

The height at BBCH 12 was significantly reduced in ‘Merlin’ and ‘Primus’, while ‘ES Mentor’ did not show a significant reduction in height when primed seeds were used, but in ‘Merlin’ and ‘Primus’ priming led to shorter plants in BBCH 12.

4.3.6 Discussion of the field experiment

4.3.6.1 Priming did not enhance emergence and early plant development

We hypothesized that priming would decrease the time to emergence. In the field study, the opposite was found: primed seeds tend to take longer to emerge, and for ‘Merlin’ this trend was significant. Contrary to the initial hypothesis, emergence was reduced by priming treatments.

The warm weather and growing conditions in the field experiments might have led to the negative effect of priming, which was also observed (arithmetically) in the former experiments in pots. In both years, the air temperature at sowing was relatively high (up to 24°C in 2016 and 22°C in 2017). In the following seven days daily mean air temperatures ranged between 14 and 18.9° (2016) and 19.9°C (2017) with soil temperatures reaching 14.5°C in 2016 and 13.6°C in 2017. Kering and Zhang (2015) found prolonged emergence after hydropriming in soybeans under dry field conditions in Virginia, USA. In the second year of that field study, with more rainfall, priming did not have a significant effect (Kering and Zhang, 2015). In contrast, faster emergence after hydropriming under dry and hot field conditions was reported in Pakistan (Arif et al., 2008) and Iran (Bejandi et al., 2009).

4.3.6.2 Mykoplant did not affect plant growth

Final emergence and the speed of emergence showed no effect of the addition of Mykoplant®. In combination with priming the seeds reacted the same as without Mykoplant® (final emergence was reduced). The Mykoplant® addition also did not show significant changes in further plant development, as no significant differences were found. A higher yield, which is excepted from the literature (Schütz et al., 2018) could not be found at all. The active ingredients in Mykoplant® are *Glomus intraradices*, *G. mosseae* and *G. etunicatum*, the two first named are quite well studied in soybean and showed increased nodulation and N fixation and improved plant biomass and yield in pot and field studies (Schmidt et al., 2015). While the yield data did not show a positive trend, shoot and root biomass, as well as nodule number showed a tendency to increase in the Mykoplant® treatment. This is contrary to the findings in the pot experiment where nodule number was not affected by Mykoplant® addition.

Besides the active AMF ingredients, Mykoplant® also contains other nutrients coming from fermented grape pomace, which is also used as organic fertilizer. It contains nutrients such as nitrate, ammonium, phosphate, potassium and trace elements. So, it is not possible to distinguish if the positive (and not significant trend) derives from the AMF or the other substances in the product.

4.3.6.3 Variety influenced plant growth

Variety affected plant growth more than the treatments did, and variety-treatment interactions were of minor relevance (just found for emergence, height at BBCH 12 and yield). Hydropriming led in all cases to a reduction or no effect. Kering and Zhang (2015) likewise reported a significant variety x priming interaction on percentage emergence depending on seed size in one of two years of field study. They reported decreased emergence in varieties with large seed sizes. It can be concluded that hydropriming tends to have a negative effect overall, but the extent depends on the soybean variety. This might be explained by different rates of water uptake and storage during and after priming.

In 'ES Mentor', the addition of Mykoplant® tended to improve germination and yield, but this could not be statistically verified. Overall variety had a strong effect, as most tested parameters were significantly influenced by variety. This shows the importance of choosing a suitable variety for the farmers' field conditions and the opportunity for soybean breeding.

4.3.6.4 Soybean can compensate for reduced emergence

The yield effects of hydropriming are reported as controversial, Helsel et al. (1986) reported reduced yields, while Mohammadi (2009) and Langeroodi and Noora (2017) found higher yields. Despite the observed overall reduced emergence in the current field trial, yield was less affected, as also reported by Ghassemi-Golezani et al. (2011). They observed that soybeans could compensate for low germination by increasing pods per plant and grains per plant. The increased number of pods found in the current field trial supports this hypothesis. Plant height and LAI were reduced with priming, so plants could not compensate for biomass production, which can leave space for weeds. In this experiment, weeds were controlled manually when needed. Therefore, it is questionable if this effect is also found under practical (only mechanical weeding) conditions. In practice, a faster and higher emergence would probably lead to higher yield due to better suppression of weeds.

The height of the first pod above the soil was also reduced due to priming treatment. These lower pods are often not harvested by combine harvesters and would not contribute to the farmer's yield. Schmidt and Langanky (2019) found a strong correlation between hand harvest and normal harvested yield, and further a correlation between the yield and the Cutting height of the combine harvester. However the cutting height is limited by soil conditions (e.g. stones and surface unevenness) (Höner, 2015).

4.3.6.5 Mechanical sowing should be tested further

Mechanical sowing of primed and dried seed could work, as the results of the pre-test show. On the edges of the experiment, priming reduced the percentage emergence to the same degree regardless of whether seeds were sown by hand or with a seed drill. This would save many working hours and make the results more comparable and usable for practical farming. Since this experiment was conducted in only one year, results must be verified.

4.4 Seed quality

4.4.1 Soak test and fungal infestation

The mean percentage of blown (= presumed damaged) seeds was 12.6% (Figure 1). The target is a percentage of less than 10% blown seeds (SDSU Extension 2012; von Beesten 2014). Only 'Opaline' could meet this criterion (6%), while 'ES Mentor' (11%), 'Merlin' (12%), 'Primus' (13%) and 'Lissabon' (21%) also had values above this threshold.

The mean fungal infection across all seed lots was 10.8%. In 'Merlin' and 'Opaline', the fungal infection was 0 and 1%, respectively (Table 21). 'Primus' had the highest infestation (25%), coming together with the second-highest number of blown seeds. 'Lissabon', which had the highest number of blown seed, had the second highest amount of fungal infection (16%).

Table 21 Results of the water soak test, done in July 2015 and fungal infestation within the germination test of the seed lots tested in November 2015, separated by variety.

Variety	blown seeds (%)	fungal infestation (%)
ES Mentor	11	12
Lissabon	21	16
Merlin	12	0
Opaline	6	1
Primus	13	25
Mean	12.7	10.8

4.4.2 Germination and emergence over time

A germination capacity of 80%, as required in the German Seed Regulation (Federal Office of Justice, 2006) was reached in the germination tests in 2015 within all soybean varieties (Figure 23).

'Merlin' and 'Opaline' achieved the best results. According to the seed supplier, the seed lot of 'Merlin' had a germination rate of 91%, in the germination tests five to 13 months after harvest 'Merlin' reached 98 -100%. After 24 months of storage (end of 2016), 'Merlin' reached still a quite good germination capacity of 94% and after 27 months of storage a germination of 75% was obtained by LfL.

'Opaline' had a germination capacity of 94%, according to the producer. In the conducted experiments, values from 92 to 100% in 2015 were found (five to 13 months after harvest). In 2016, the germination declined to 64% after 16 months of storage and 55% one month later.

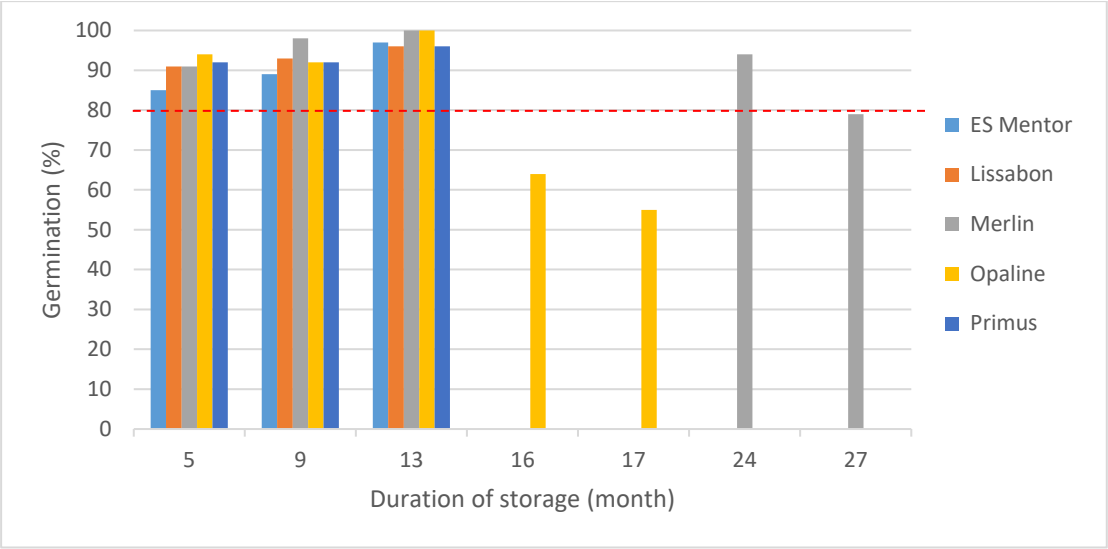

Figure 23 Germination capacity over time. The red line marks the required minimum germination capacity by the German Seed Regulation for soybeans.

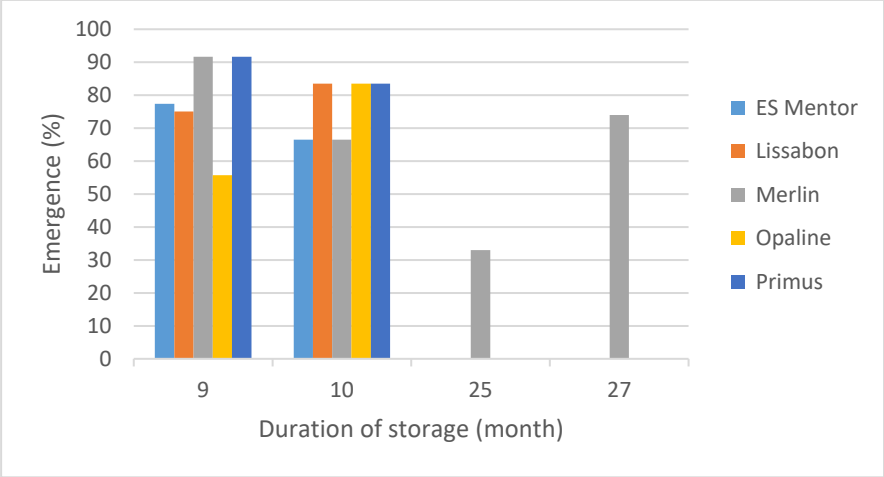

Figure 24 Emergence of soybean seeds over time

The field emergence is usually lower than the germination tested in the laboratory. The mean emergence rate reached 78% after nine months and 77% after ten months of storage. The values of the different varieties showed greater variation within the emergence than within the germination. This might be due to the different number of seeds tested, which was 100-200 seeds for each soybean variety in the germination test, while only six to 36 seeds each were used in the emergence test.

After two years of storage, the emergence tests were repeated with the variety ‘Merlin’, which resulted in an emergence rate of only 33%. The subsequent LfL cold test two months later gave comparable normal results of 74%.

4.4.3 Germination and emergence depending on temperature

The germination and emergence of plants rely on the ambient temperature. An ambient temperature of 25°C resulted in 96% of germination, this declined to 92% at 22°C. At 18°C, the overall emergence was 85%, declining to 66% at 12°C.

The soybean varieties reacted differently to the declining temperatures. 'ES Mentor' was the most successful variety at 18 and 15°C, while under 12°C 'Merlin' showed an emergence rate of more than 90%. Also, within the germination test at 22°C, 'Merlin' showed best results. 'Merlin' is a standard variety under cold growing conditions (Zimmer et al., 2016) and belongs to the maturity group of '000' (very early). The other very early variety ('Lissabon') showed a less successful emergence under suboptimal growing conditions but had also the highest number of blown seeds (21%). 'Opaline', maturity group '00/000' (between early and very early) showed second best results under germination under 22°C and emergence under 18 and 15°C, but the emergence rate was reduced to under 50% at 12°C.

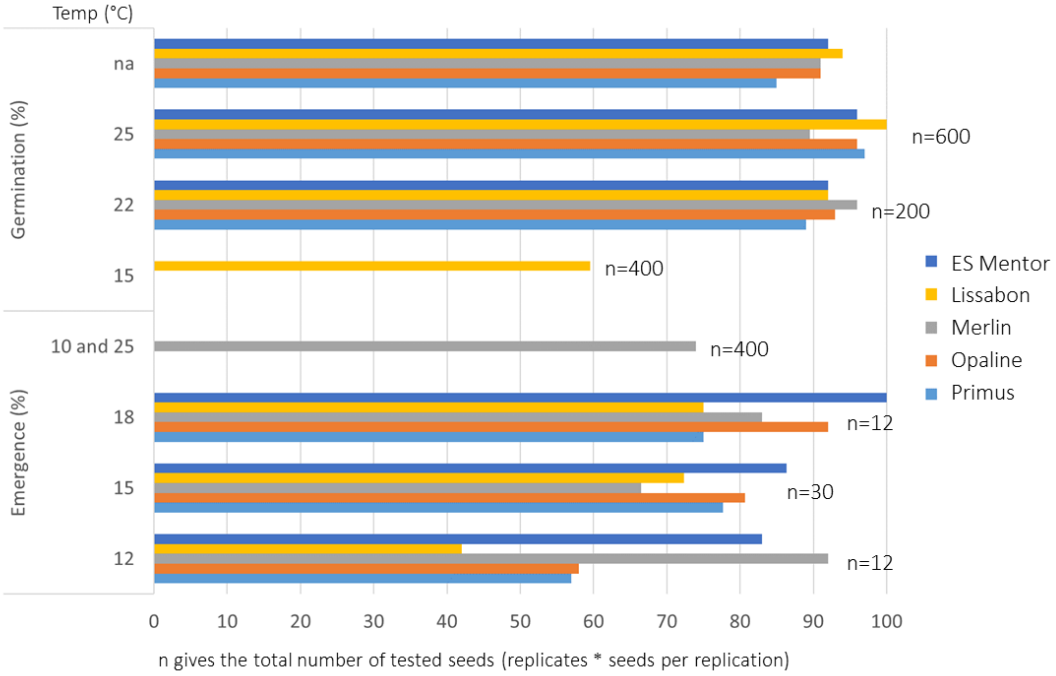

Figure 25 Germination and Emergence in dependence of the temperature while testing seed quality

4.4.4 Discussion on seed quality testing

The very low emergence success (33%) of 'Merlin' under 15°C after 25 months of storage can partly be explained by the temperature of 15°C. Also, in July 2015 (after nine months of storage), 'Merlin' showed the lowest result under 15°C, compared to 18°C as well as to 12°C. Tests by LfL reported a germination rate of 79% and a field emergence (examined with the cold test) of 74%, they concluded, that a constant temperature of 15°C is too cold for the seeds to grow.

4.4.4.1 Seed quality

'Merlin' and 'Opaline' showed the highest germination. These varieties were also the ones showing the lowest number of seed coat damages (as indicated by blown seeds) and the lowest fungal infestation. This is in line with other authors (Haikal, 2008; McGee, 1980) since high fungi incidence can reduce germination by about 13% (Costa et al., 2013). Soybeans are very sensible towards injury and damage of seed coat at handling, e.g., during harvest, post-harvest handling, drying etc. (Shelar et al., 2008). Also, internal mechanical damages are reported (Ning et al., 2014). The water soak test can assist in evaluating the seed quality, although it is not directly linked to the current seed germination. After one year of storage, the seed germination outcome was consistent with the results of the soak test. Farmers are suggested to conduct this test in the field to ensure appropriate machine settings for harvesting and handling seeds for propagation, avoiding damage to the seed coat, and thus maintaining seed quality (von Beesten, 2014).

A negative trend in germination and emergence rate with increasing storage duration could neither be observed nor excluded during the experiment. The different dates are not fully comparable in terms of sample size, temperature and the used methods. The seeds were stored in conditions (~ 4 to 8°C and 30-52% relative humidity), as advised in literature: cold and low relative humidity (Abbasi Surki et al., 2012; Miersch, 2015). Balesevi et al. (2011) found a higher germination rate in seeds stored at 4°C and 80-85% relative humidity as compared to uncontrolled storage conditions in Serbia. However, even under controlled conditions, the storage reduced the germination rate to 81.3% after six months and 72% after 12 months. Sheidaei et al. (2014) found germination rates of 85% after harvest, 80% after six months, 75% after 18 months and 65% after 30 months of storage. This is in line with other studies showing declining viability of soybean with increasing storage ((Coradi et al., 2020; Sheidaei et al., 2014) even under good storage conditions (Shelar et al., 2008), but especially under natural (and thus varying) conditions (Balešević-Tubić et al., 2010; Mbofung et al., 2013). Compared to other crops soybean is more susceptible to storage viability losses (Balešević-Tubić et al., 2010).

4.4.4.2 Which method to choose for testing soybean seed quality

As described above fungi incidence reduces germination of soybean. A survey of germination testing laboratories in 2012 showed that the disinfection of seeds before germination testing is not standardized, e.g. 24% of the 122 laboratories reported that they disinfect certain seed species as a matter of routine, including soybean seeds (Don, 2013). The most important fungal disease is Diaporthe/Phomopsis (Bachteler, 2018): These fungi infect the seed before harvest in moist and warm weather conditions. The infection starts at the seed coats and continues towards the inner seed parts. Depending on the duration of the infection the seed coat or the seed itself is infected (Franca Neto and West, 1989). While infected seed coats led to high infection rates (and low germination) in paper-

based germination tests, germination tests on sand and soil are less susceptible to fungal infection and results are closer to observed field emergence (Franca Neto and West, 1989). It is assumed that the infected seed coat is left behind in the soil, while on paper it stays on the seedling and the fungal infection continues (Franca Neto and West, 1989). They advised to standardize the seed emergence test since the rolled-paper-towel test was not suitable for soybean seeds infected with Phomopsis spp.

The germination test on (pleated) paper is meanwhile no longer common for soybeans in laboratories in Germany working after ISTA standards (Killermann and Voit, 2016). Instead, the method on sand is used more often. In their annual meeting in 2021, ISTA included a medium for soybean tests, the "crepe cellulose paper", so laboratories still using germination tests for soybeans based on paper (Johnston, 2021). The ISTA test on germination capacity is done at 25°C, which is the optimal growing condition for soybeans. Instead, the LfL cold test applies a combination of seven days under cold conditions (10°C) followed by seven days at optimal (25°C) growing conditions. According to Schmidt, Voit and Killermann (2016) this gives a prediction of the minimal germination rate under suboptimal field conditions. The expected field emergence of the seed lot should be between these two values. Depending on the research question, the LfL cold test could be a better method of choice, since it imitates the cold temperatures at soybean sowing.

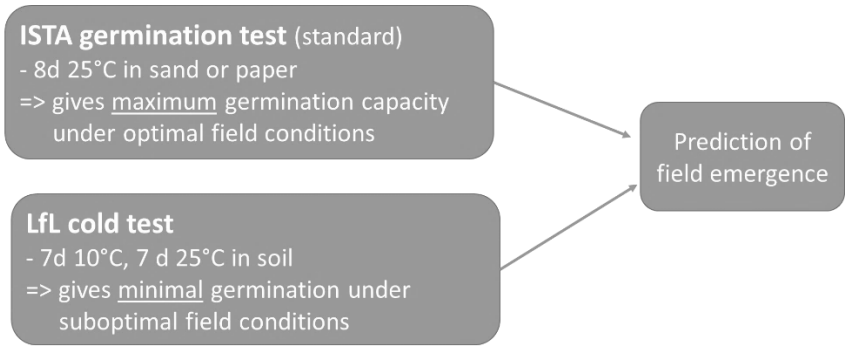

Figure 26 Prediction of field emergence using ISTA and LfL cold test, as described by Schmidt, Voit and Killermann (2016)

5 Synthesis

In this chapter, the results and insights from the various experiments will be combined and placed in a broader context with the literature to explain the results and point the way forward.

5.1 Effect of hydropriming on soybean germination and emergence

5.1.1 No improvement with priming

The various experiments revealed a decrease in final germination or emergence, with minimal or no improvement in the time needed to germinate and emerge (Figure 27).

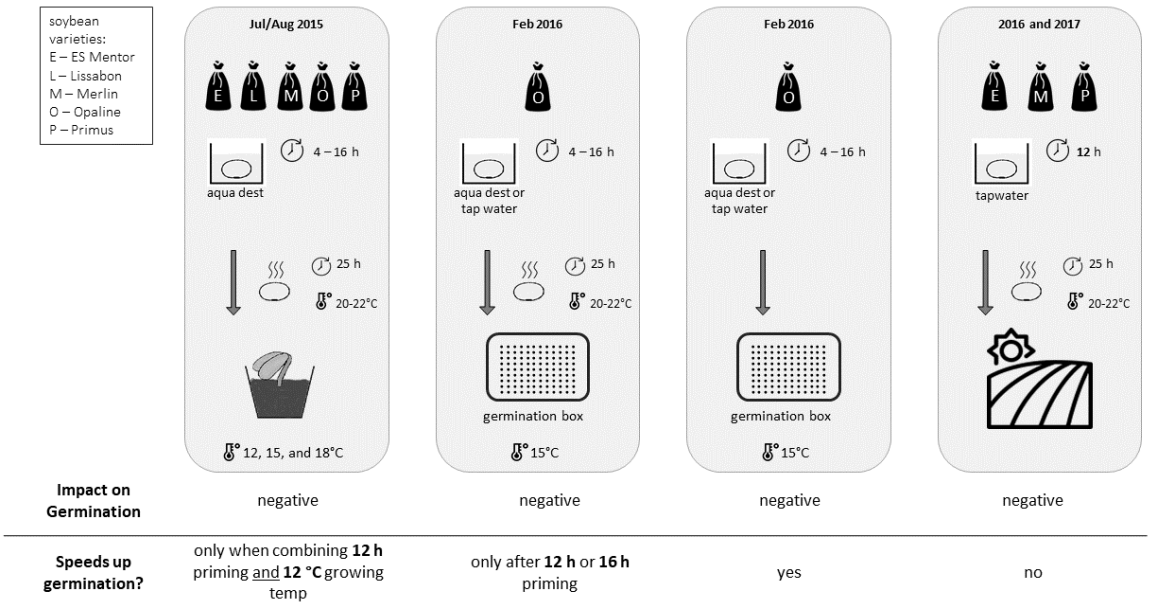

Figure 27 Summary of all experiments concerning hydropriming. Own illustration, using own icons and icons of bag, clock, thermometer and field used from icons8.com

Different priming durations and soil temperatures were tested in pot experiments, leading to an average decrease in emergence from 79% in control treatments to 65% in hydroprimed treatments. In the field experiment, the reduction in emergence was even more pronounced, dropping from 84% to 57%.

In the pot experiments, the time to germinate was only reduced after 12 h priming within the 12°C climatic chamber, for approximately 2 days. This is lower than the reported decrease of 2.4 hours in mean germination time for each hour of priming by Bharati et al. (1983). It is worth noting that the temperature during priming is also a vital factor, as highlighted by Corbineau et al. (2023), and should always be mentioned in studies. In the present study, the temperature during priming was not

regulated. However, the priming containers were positioned within the laboratory with a constant room temperature of approximately 20-22°C.

There is inconsistency in the literature regarding the exact type of water used. Some studies report on using distilled water or tap water, of which the quality differs among regions, while others do not specify the type of water used. In the current study, distilled water and tap water were compared in germination tests. No significant difference was found. For practical reasons, tap water was chosen for the field experiment.

During the germination tests, primed seeds showed a faster emergence speed when seeds were not dried subsequently. They germinated approximately 2.5 days faster than the control group. However, this effect was not observed when the seeds were dried for 25 hours after priming. The seed industry typically dries seeds before storing them (Corbineau et al., 2023), and it can be assumed that the drying conditions affect the emergence success, which may vary among crops and even varieties. Further research should consider drying options to achieve a moisture level appropriate for storage.

For the field experiments, 12 h priming was chosen, as it showed under 12°C in the pot experiments a faster emergence. In the field, no faster emergence was found, but total germination was reduced.

5.1.2 What determines the success of hydropriming?

The reduction in germination capacity found in the current study raised questions concerning the factors contributing to successful hydropriming in soybeans. Therefore, the literature collected during the project that investigated hydropriming in soybeans and provided data on final germination and emergence was thoroughly evaluated. This involved a detailed analysis of 24 studies that primarily focused on hydropriming and those, which tested different priming methods including hydropriming. The studies were categorized according to their effect on germination or emergence. Details of the priming method and outcome of the study were summarized (refer to Annex Table 31, Table 32, and Table 33 for more information).

A decrease in soybean germination and/or emergence has also been noted by other authors, both under field conditions (Ghassemi-Golezani et al., 2011; Helsel et al., 1986; Kering and Zhang, 2015; Miladinov et al., 2018a; Aminu et al., 2022), in laboratory (Sibande et al., 2015) and pot conditions (Weerasekara et al., 2021). However, most studies suggest that hydropriming treatments enhance soybean germination and emergence. The majority of experiments were conducted under standardized laboratory conditions, with a temperature of 25°C, using either silt or paper substrates (Maroufi and Farahani, 2011; Arif et al., 2014; Kujur and Lal, 2015; Mohamed et al., 2018). However, some studies employed lower germination temperatures, such as 10°C (Bharati et al., 1983; Moosavi

et al., 2014), while others examined a range of temperatures spanning from 5 to 35°C (Mohammadi, 2009). Field studies have also reported that hydropriming treatment can enhance emergence (Arif et al., 2008; Moosavi et al., 2012; Miladoniv et al., 2014; Langeroodi and Noora, 2017).

Most studies originating from Iran (Mohammadi, 2009; Bejandi et al., 2009; Maroufi and Farahani, 2011; Moosavi et al., 2012, 2014; Langeroodi and Noora, 2017; Ghassemi-Golezani et al., 2011; Rouhi et al., 2011; Moshtaghi-Khavaran et al., 2014), where soybean is planted as a second crop in very late spring (Mohammadi, 2009). In recent years several studies were conducted in Serbia by Miladinov and colleagues (Miladinov et al., 2015, 2018b, 2018a, 2019, 2020, 2021).

Only six studies focused on **growing temperatures** below the optimal of 25°C. Of these, two found a negative impact of priming (Helsel et al., 1986; Ghassemi-Golezani et al., 2011), one found no impact (Moshtaghi-Khavaran et al., 2014), and three found an improvement through hydropriming (Bharati et al., 1983; Mohammadi, 2009; Moosavi et al., 2014). Field studies showed a higher proportion of negative impact on germination compared to laboratory studies. While 29% of all studies examined demonstrated an adverse effect, 38% of field studies displayed unfavourable results (Figure 28).

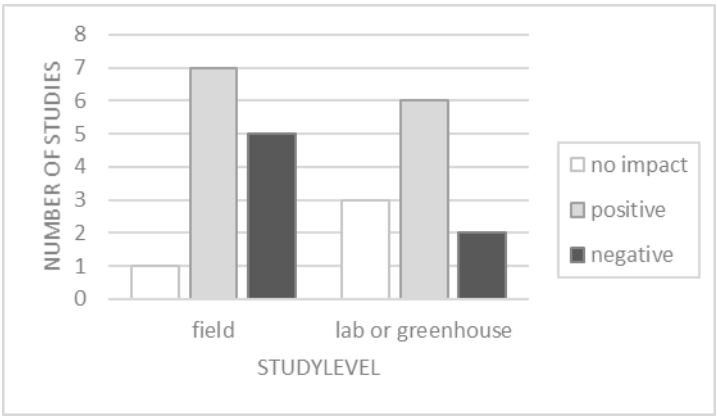

Figure 28 Study outcomes in relation to the study level

The importance of oxygen: The absence of oxygen may have had negative effects on the current hydropriming experiments as the seeds were fully submerged in water without any aeration. Corbineau and Côme (2006) suggest that priming conditions, including oxygen concentration and temperature, should mimic those facilitating optimal germination. Some of the studies that investigate hydropriming could be categorised as solid matrix priming. This is because soaking the seeds in water was not carried out, instead, wet paper towels were used (Assefa and Hunje, 2010; Costa et al., 2013; Kujur and Lal, 2015; Mohamed et al., 2018). Two of these studies demonstrated a favourable influence on seed germination in laboratory conditions (Kujur and Lal, 2015; Mohamed et al., 2018), while two indicated no effect, one within a laboratory setting (Costa et al., 2013) and the other in a field environment (Assefa and Hunje, 2010). Two other studies investigating hydropriming implemented

aeration, one of which used an aquarium pump for this purpose (Arif et al., 2014), while the other did not specify the method of aeration (Bejandi et al., 2009). Arif et al (2014) reported an increment in germination after six hours of priming, but observed a decline with longer priming periods. Bejandi et al (2009) documented elevated germination rates, faster emergence and greater grain yield in field conditions in Iran when seeds were primed. None of the studies that utilised solid matrix priming detected a negative impact, indicating a potential oxygen deficiency.

Improved germination was observed in tomato plants after priming in aerated water compared to non-aerated water and moistened paper (Santika et al., 2022). Aeration plays a crucial role in achieving successful priming (Corbineau and Côme, 2006). To ensure sufficient priming success, oxygen content must be at least 5% (Corbineau et al., 2023). However, the species-dependent effect should be taken into consideration (Raj and Raj, 2019).

Seed quality influences the effectiveness of hydropriming. When clustering for different seed quality groups (Figure 29), studies suggest that hydropriming is more advantageous for those with low initial germination capacity. However, studies using high-quality seeds (80-100% germination in the control) show more diverse results. Out of the nine studies conducted with high-quality seeds, three indicated an increase in germination/emergence, three indicated a decrease, and three showed no difference. This aligns with studies focusing on this issue, e.g. Miladinov et al. (2021) found a positive effect of priming on older seeds with reduced initial germination. Tamindžić et al. (2023) report an increase in the final germination of seeds with lower initial germination in Pisum sativum L. Priming triggers the initial stages of seed germination, involving specific metabolic changes such as DNA repair activation (Paparella et al., 2015). This effect may be more advantageous for low-vigour seeds than high-vigour seeds.

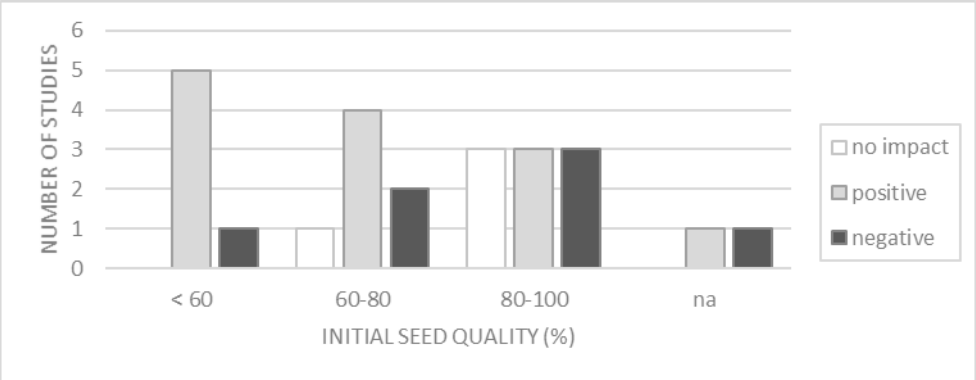

Figure 29 Study outcomes in relation to initial seed quality (germination %)

Number of soybean varieties under study matters. Only a small number (five) of the 24 studies examined in this research included experiments with more than two types of soybean seeds (Bharati et al., 1983; Helsel et al., 1986; Kering and Zhang, 2015; Miladinov et al., 2018a; Aminu et al., 2022).

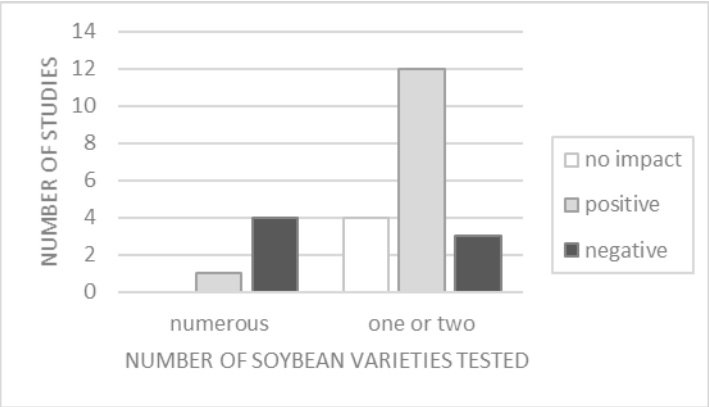

Figure 30 Study outcome in relation to the number of soybean variety tested

Among these studies, four revealed a decrease in germination and/or emergence following hydropriming treatment (Figure 30), which mirrors the findings of the present study. However, Bharati and colleagues (1983) discovered an enhancement in germination under cold conditions. No significant interaction between hydropriming and soybean variety was found in the current study. However, other authors have reported about variety-specific reactions. Lewandowska et al. (2020) discovered a genotype-specific reaction to solid matrix priming under cold spring conditions, even when the varieties originated from the same maturity group. Kering and Zhang (2015) observed that priming resulted in decreased germination in soybean varieties with larger seeds. However, even among single varieties, seed size plays a significant role in determining the germination process. Pereira et al. (2013) discovered that larger soybean seeds showed better germination under optimal moisture conditions, but under water deficit, smaller seeds of the same variety performed better (Hoy and Gamble, 1987).

The focus of the research paper. Another notable discovery was made regarding the research focus. When hydropriming was utilized as a control group and was not emphasized in the title, it demonstrated a lesser ability to advance seed germination or emergence in comparison to the studies that mentioned hydropriming in the title (Figure 31). This could be indicative of publication bias; however, since the literature search did not follow the PRISMA method (Page et al., 2021) systematically, this effect should not be exaggerated. This may constitute a subject for a future meta-analysis.

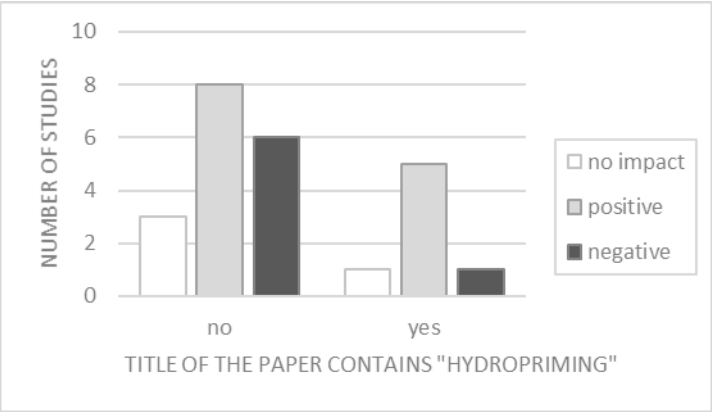

Figure 31 Comparing study outcomes of hydropriming, separated if the study title contained "hydropriming" or not.

5.1.3 Constraints and limitations of the studies on hydropriming

The pot experiment on hydropriming consists of four replications, each holding three seeds. From the current perspective, this number of plants seems to be too low. Higher number of plants per replication, e.g. 100 seeds each seems to be more robust.

The literature review about hydropriming in soybean is not done systematically, since it used the literature that was already collected during the whole duration of the research project, supplemented with an additional literature search. The better option would be to use a standardized literature search and report, as the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement describes (Page et al., 2021). However, due to a lack of time, and difficulties finding common keywords which also include the studies which used hydropriming only as a control group, it was decided to use the already available material instead of conducting a whole new study.

5.1.4 Alternatives to hydropriming

The study aimed to achieve quicker and more synchronized germination through hydropriming in order to suppress weeds, which aligns with breeding objectives (Horneburg et al., 2017). Seeds from the same seed lot have different germination times (Moshtaghi-Khavaran et al., 2014). This heterogeneity leads to a lack of synchronised germination performance, especially under stressful environmental conditions at sowing (Corbineau and Côme, 2006). Probably, the germination process (lag phases I to III) does not occur uniformly across all the seeds within a seed lot. In such circumstances, a specific duration of hydropriming would not synchronize seeds, as opposed to an osmotic condition or solid matrix priming.

Several studies have investigated soybean pre-treatments, comparing hydropriming to alternative priming techniques. These studies have demonstrated that osmopriming leads to faster germination

or emergence and higher total germination rates (Helsel et al., 1986; Arif et al., 2008, 2010; Rouhi et al., 2011; Langeroodi and Noora, 2017). Additionally, hormonal priming (Bejandi et al., 2009; Langeroodi and Noora, 2017) and priming in mineral solutions (Mohammadi, 2009) have also been shown to be effective methods. Numerous other priming techniques exist, including solid matrix priming, biopriming, chemopriming, halopriming, thermopriming and drum priming (Ashraf and Foolad, 2005; Paparella et al., 2015). Mohammadi's (2009) study examined various priming media in both field and laboratory conditions and concluded that priming with potassium nitrate, ammonium nitrate, or calcium nitrate produced superior outcomes compared to hydropriming or priming with sodium nitrate.

Solid matrix priming, utilizing wet paper for priming (Kujur and Lal, 2015) or wet sand (Lewandowska et al., 2020) may lead to improved aeration, and thus, better germination and emergence. This may be because imbibition is slower in all other priming treatments compared to hydropriming. Fast imbibition can trigger DNA degradation and diminish soybean seed performance, especially field emergence (Pereira and Masetto, 2021).

Combining priming and the addition of beneficial microorganisms, known as biopriming (Jisha et al., 2013), provides an opportunity to improve germination whilst controlling for pathogens or incorporating other plant growth-promoting advantages (Corbineau et al., 2023). For instance, biopriming soybeans with *Pseudomonas aeruginosa* reduced damping-off caused by *Colletotrichum truncatum* and enhanced seed germination (Begum et al., 2010).

In addition to priming, several pre-sowing methodologies have been successfully tested in soybeans. For instance, Lewandowska et al. (2019) discovered positive effects of static magnetic fields. Another technique is Cold Atmospheric Pressure Plasma, which increases germination (Ling et al., 2014) and leads to faster water uptake within the first hour of imbibition (Svubova et al., 2021).

With climate change, sowing dates will also change: Kaukoranta and Hakala (2008) reported for west Finland a significantly earlier sowing date of 3.1 days per decade, measured for different crops in the period 1980-2007. Models for Europe indicate that maize (which is also sown late and thermophilic as soybean) could be sown 6 – 23 days earlier in 2040 (Olesen et al., 2012). A prolongation of the vegetative phase in combination with early maturing varieties and seed pre-treatments can enhance soybean adaptation in Germany.

5.2 Seed additives to improve soybean germination and growth

5.2.1 No improvement through seed additives

The addition of the seed additives under study did not improve early plant development. The addition of arbuscular mycorrhizal fungi (AMF) using Mykoplant® to the seeds in the pot experiment led to an improvement in the early stages of germination, but this was offset when the first leaves were fully developed (BBCH 12). In the field experiments, Mykoplant® did not improve final yield or early plant development (Figure 31).

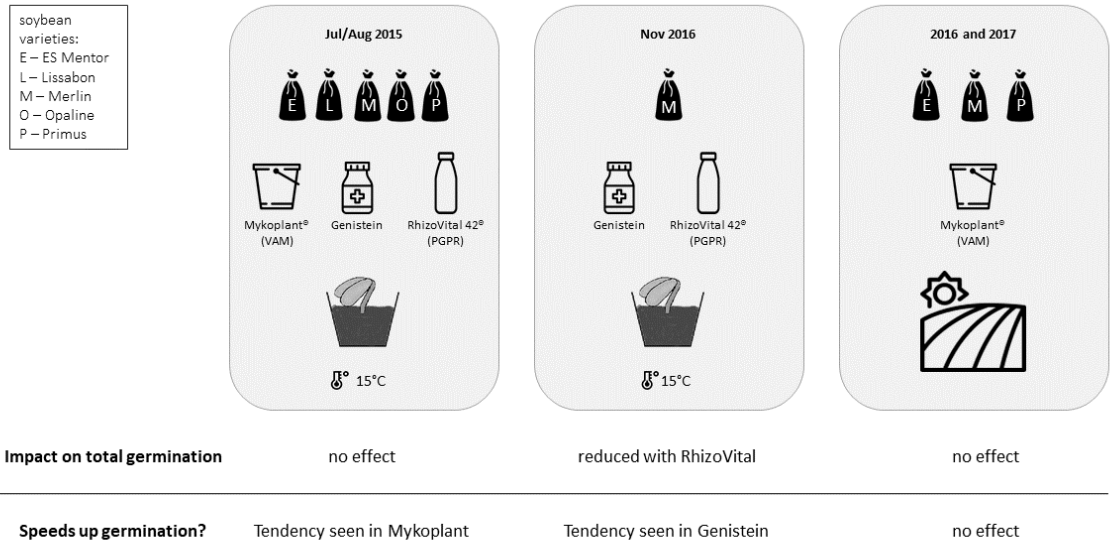

Figure 32 Summary of all experiments concerning seed additives. Own illustration, using own icons and icons of bag, clock, thermometer, bucket, pill box, bottle and field used from icons8.com

Most studies report improvements in later plant development with AMF and PGPR. Research focusing on emergence has shown promising results (Kloepper and Scher, 1987). Alahdadi et al. (2009) found better germination and seedling vigour after biofertilization with *Pseudomonas fluorescen* and *Glomus mosseae*. The use of *Bacillus spp*. was found to improve germination while showing antifungal activity against *Diaporthe caulivora*, followed by *Diaporthe sojae*, *Diaporthe eres*, *Diaporthe longicolla* and *Macrophomina phaseolina* (Miljaković et al., 2022).

The use of Mykoplant® tended to increase nodule size in pot experiments, while in the field more but smaller nodules were observed when Mykoplant® was used. Both observations were not statistically proven. Other authors also show different effects on root nodules (in cowpeas) when co-inoculated by *Bradyrhizobia* and AMF (Pereira et al., 2020). Nodule formation requires high P availability in plants, which can be met when there is a symbiosis with AMF (Meena et al., 2018; Yadav et al., 2013). But

under low root zone temperatures, mycorrhizal colonisation showed a negative effect on nodule number (Zhang and Smith, 1995). The combination of *B. japonicum* with the free-living PGPR *Azospirillum brasilensense* has shown mixed results, with Cassán (2009) finding an increase in nodule number in soybean and Hungria (2015) finding no effect on nodule number. Mortimer et al. (2008) reported about suppressed nodulation by AMF colonization in *Phaseolus vulgaris* seedlings.

5.2.2 Potential antagonistic effects

In the pot experiments, no improvement was observed with the added products, but the addition of all three products (and *Bradyrhizobia*) reduced the plant traits studied. Previous studies have shown reduced nodule development at low temperatures (Zhang and Smith, 1994). The addition of genistein was found to increase nodule mass but without increasing nitrogen formation. The authors concluded that nodules formed at suboptimal root zone temperatures would be less efficient (Zhang and Smith, 1995). However, the combination of pre-incubation of genistein with *Bradyrhizobia* and the addition of other PGPGs can have negative effects at low temperatures (Dashti et al., 2014; Zhang et al., 1997). This is consistent with the significant reduction in plant development in the current study when all three products were added to the seeds. Genistein led to an earlier onset of nodulation (Zhang and Smith, 1995). The addition of other symbiotic partners requires the young plant to provide different partners with C-assimilates. In later stages, the plant responds to this increased demand by increasing leaf area to supply the symbiotic partners (Harris et al., 1985). At this early stage of development, this increased demand may explain the reduced plant development when all products are combined.

5.2.3 Constraints and limitations of the studies on seed additives

Also, as in the hydropriming experiments, the results of the pot experiment for seed additives should be interpreted with caution as it consisted of only three replicates, each containing one plant. The research design planned at least six plants, but this could not be achieved due to limited access to the climatic chamber. Six replicates would be better, but still seems too few at this stage. Another limitation is the use of products rather than strains. The reason for this was to make the results more transferable to farmers, but the effect of the strain cannot be separated from the effect of the other nutrients in the products.

Another limitation is that the phosphorus status of the soil due to different circumstances was not analysed, which would be of interest when interpreting the AMF data. It is known that the synergistic relationship between AMF and rhizobia depends on the amount of plant available P and N (Wang et al., 2011).

5.2.4 Future perspectives

A huge number of different PGPRs and AMFs are being studied (Schütz et al., 2018), and with new gene-based technologies, more soil microbes will be identified (van der Heijden et al., 2015). The effect of PGPRs and AMFs depends on the current soil biotic state, which is shaped by numerous factors such as soil and climatic conditions, as well as crop rotation (van der Heijden et al., 2015) and management type (organic or conventional) (Mäder et al., 2000; Gitonga et al., 2021). As a result, the microbiome is different and so is the effect of PGPR and AMF inoculation, as they must compete with native strains in the soil (Zeffa et al., 2020). New technologies can not only assist in the extraction and use of strains but also allow the effect of microbial addition to natural soil biota to be studied (Cozzolino et al., 2021).

The effect of a particular microbial agent on different host plants is not always equally effective or may even be negative (Chen et al., 2018). More research is needed to screen for suitable host-specific microbial agents (Zahir et al., 2003). Schmidt et al. (2015) identified strains of *Pseudomonas, Bacillus* and *Azospirillum* as the most effective for soybeans under cold growing conditions. A meta-analysis found *Bacillus* to be the best co-inoculation partner for *B. japonicum*, with 33% more nodules found under field conditions, but no significant effect on plant nitrogen content or yield (Zeffa et al., 2020). Li et al. (2022) also found *Burkholderia* to be very effective on legumes, resulting in yield increases. As soybean variety and PGPR show interactions (Zimmer et al., 2016), this should be taken into account when researching the most suitable microbial-host combinations. As PGPR also have great potential to improve plant health and suppress certain diseases (Miljaković et al., 2022), this potential should also be considered.

5.3 Seed quality testing

Soybean seed is more sensitive to overstocking than other crops (Shelar et al., 2008). This needs to be considered when planning germination experiments with soybeans. Seed availability limits research in the laboratory or greenhouse if experiments are carried out outside the normal sowing period, the seed is difficult to obtain, and the length of storage will affect results. Seed should be used as fresh as possible, even if different seed lots are used for different tests. It is important to control (and document) storage conditions and to check germination capacity immediately before the trials to ensure that seed quality is maintained.

All tests should use a large number of seeds (50 seeds or more) to reduce the single-seed effect and provide robust data. Germination tests should be carried out in sand (in large boxes) to avoid fungal infection affecting the results. For field data, the 'cold test' (Voit, 2016; Voit et al., 2012) is recommended.

6 Conclusions

This research aimed to identify an effective method of improving soybean emergence under cold growing conditions that could be used by farmers, particularly in Germany. The main questions were whether hydropriming can improve soybean emergence under various conditions and whether seed additives (PGPR, AMF and genistein) can improve soybean emergence under cold conditions.

Based on pot experiments, germination tests and a field study, it can be concluded that hydropriming does not work for the studied soybean varieties, at least in the way it was used in this study. With regard to seed additives, it was found that the products studied had no effect (except inoculation with *B. japonicum*, which is common practice). Due to the small sample size and limited number of products, the results cannot be generalised. This also raises the question of powerful and meaningful germination experiments for cold growing conditions.

The results showed no interaction effects of soybean variety with treatments or products. Based on this, further screening studies for effective methods in soybeans could be carried out with one variety and the results subsequently verified with more varieties.

This research attempted to transfer hydropriming, a well-described method for improving emergence and plant vigour for various crops, to soybeans under German climatic conditions. Rather than establishing a protocol that could be used by farmers or seed companies, questions were raised as to whether this method would work in soybeans at all and under which conditions.

Further research is needed to find suitable PGPR and AMF for (co-)inoculation in soybeans. As this study shows a negative effect of hydropriming on seed germination, other priming techniques should be used. To obtain robust data and results suitable for cold growing conditions, emergence tests should include a large number of seeds at cold temperatures.

7 References

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8 Annex

Table 22 Microbial agents found in the products on the German market and their type of action. The grouping of N fixers is done according to Schütz et al. (2018)

Microbial agents	**MO Group**	**Number of products**
Gigaspora margarita	AMF	1
Glomus aggregatum	AMF	2
Glomus brasilianum	AMF	1
Glomus clarum	AMF	1
Glomus Clarus	AMF	1
Glomus Deserticola	AMF	2
Glomus etunicatum	AMF	4
Glomus Fasciculatum	AMF	1
Glomus monosporus	AMF	1
Funneliformis mosseae (former known as Glomus Mosseae)	AMF	6
Pisolithus tinctorius	AMF	1
Rhizophagus irregularis (former known as Glomus intraradices)	AMF	8
Rhizopogon amylopogon	AMF	1
Rhizopogon fulvigleba	AMF	1
Rhizopogon luteolus	AMF	1
Rhizopogon villosulus	AMF	1
Azospirillum brasilense	PGPR (N fixers)	1
Azotobacter chroococcum	PGPR (N fixers)	1
Bacillus subtilis	PGPR (N fixers)	1
Bradyrhizobium japonicum	PGPR (N fixers)	7
Rhizobium leguminosarum biovar viceae	PGPR (N fixers)	1
Penicillium bilaii	PGPM	3
Trichoderma asperellum	PGPM	1
Trichoderma harzianum	PGPM	2

Trichoderma koningii	PGPM	1
Bacillus amyloliquefaciens	PGPR	4
Pseudomonas fluorescens	PGPR	2
Bacillus megaterium	PGPR	2

8.1 Additional material for priming tests

Table 23. Tap water quality analysis report from water supply company done in July 2021

Parameter	Unit	Test results
pH-Value		7.90
electrical conductivity at 25° C	µS/cm	342
Oxygen (O_2)	mg/l	7.52
Anions		
Chloride (Cl^-)	mg/l	19.9
Fluoride (F^-)	mg/l	< 0.15
Hydrogen carbonate (HCO_3^-)	mg/l	149
Nitrate (NO_3^-)	mg/l	26.3
Sulphate (SO_4^{2-})	mg/l	12.3
Cations		
Calcium (Ca^{2+})	mg/l	38.4
Potassium (K^+)	mg/l	1.23
Magnesium (Mg^{2+})	mg/l	16.4
Sodium (Na^+)	mg/l	6.06
Elements and heavy metals		
Aluminium (Al)	mg/l	<0.02
Phosphorus (P)	mg/l	<0.01
Silicon compounds (Si)	mg/l	16.8

Table 24. Germination characteristics in germination test according to Ranal (2009). G: Germination, $\bar{t}$: mean germination time, CV: Coefficient of variation of germination time, $\bar{v}$: mean germination rate, U: Uncertainty, Z: Synchronization index.

Medium/treatment		N=	G (%)	$\bar{t}$ (day)	CV	$\bar{v}$ (day^{-1})	*U* (bit)	Z
Control		8	59.5	17.12	20.92	0.059	2.99	0.125
Distilled water + dried	mean		25.6	16.93	17.76	0.060	2.58	0.112
	4	4	25.5	18.15	17.42	0.055	2.39	0.153
	8	4	21.5	17.64	18.79	0.057	2.68	0.062
	12	4	30.0	16.00	17.54	0.063	2.72	0.108
	16	4	25.5	15.95	17.30	0.064	2.54	0.127
Distilled water	mean		25.6	14.65	23.22	0.069	2.67	0.121
	4	4	26.0	14.84	19.57	0.068	2.67	0.113
	8	4	26.0	14.21	26.64	0.071	2.82	0.106
	12	3	24.0	15.58	25.41	0.064	2.85	0.079
	16	4	26.0	14.19	21.81	0.071	2.36	0.175
Mineral water	12	4	28.0	13.62	20.78	0.074	2.59	0.135
Tap water	mean		24.5	14.65	24.94	0.069	2.58	0.122
	4	3	24.7	14.45	20.49	0.069	2.67	0.108
	8	4	23.0	15.27	32.53	0.066	2.44	0.136
	12	4	24.5	14.08	21.42	0.071	2.56	0.129
	16	4	26.0	14.75	24.20	0.068	2.69	0.113
Mean			30.2	15.55	21.67	0.065	2.66	0.120

Table 25: F and p-values by ANOVA for the effect of soybean variety, seed priming duration and temperature in climatic chamber on MTE and percentage emergence in pot experiment.

source of variation	**df**	**MTE**		**Emergence**	
		F	*p*-value	F	*p*-value
Variety	4	29.73	< 0.001	22.91	< 0.001
Priming duration	4	2.30	0.060	6.42	< 0.001
Temperature	2	992.10	< 0.001	0.68	0.510
Variety-Priming duration	16	1.36	0.164	1.07	0.381
Variety-Temperature	8	2.86	0.005	0.68	0.713
Priming duration-Temperature	8	2.31	0.021	0.90	0.517
Priming-Variety-Temperature	32	1.09	0.354	0.81	0.751
Equal variances		no		no	
Normal distribution		no		no	
N		281		300	

8.2 Additional material seed additives

Table 26 Product properties of Mykoplant ® 100 BT-H

- Blähtongranulat 2 –4 mm
- **Fermentierter Traubentrester**
- VAM Pilzarten Glomus intraradices, Glomus etunicatum, Glomus mosseae
- VAM Aktivität : 110.000 IE/l (IE=Infektive Einheiten)

Weitere Bestandteile:

- Nährstoffe (mg/l): Nitrat: 2,4
- O-Phosphat: 0,07
- Gesamtphosphat: 1,2
- Kalium: 2,6
- Ammonium: 0,17

Makronährstoffe im Traubenstrester-Anteil (%):

- Gesamtstickstoff: 1,2-1,9
- Phosphat: 0,2-0,5
- Kalium: 1,2-1,9
- Calcium: 0,8-1,2
- Magnesium: 0,15-0,18

Spurenelemente (mg/Kg):

- Bor: 18-22
- Eisen: 900-1.200
- Kupfer: 13-17
- Mangan: 40-45
- Molybdän: 1,1-1,6
- Zink: 32-36

Sonstige Merkmale:

- PH-Wert: 8,5
- Leitfähigkeit: 197 µS/cm
- Nitrit: < 0,01 mg/l
- Spez. Gewicht: 400 – 450 g/l

8.3 Additional material field study

Table 27 Management of the field trail in both years

Agricultural measure	**2016**	**2017**
Plough	10.03.	21.12.2016
Preparation of seed bed		
1.	29.04.	31.03.

2.	02.05., 04.05.	19.04.
3.	06.05.	11.05.
Sowing	06.05.	11.05.
Maintenance		
Netting	13.05. – 20.06.	17.05. – 06.06.
Hand weeding	06.06., 21.06., 13/14.07.	28.06.
Machine weeding	20.06., 01.07.	24.05., 10.06.

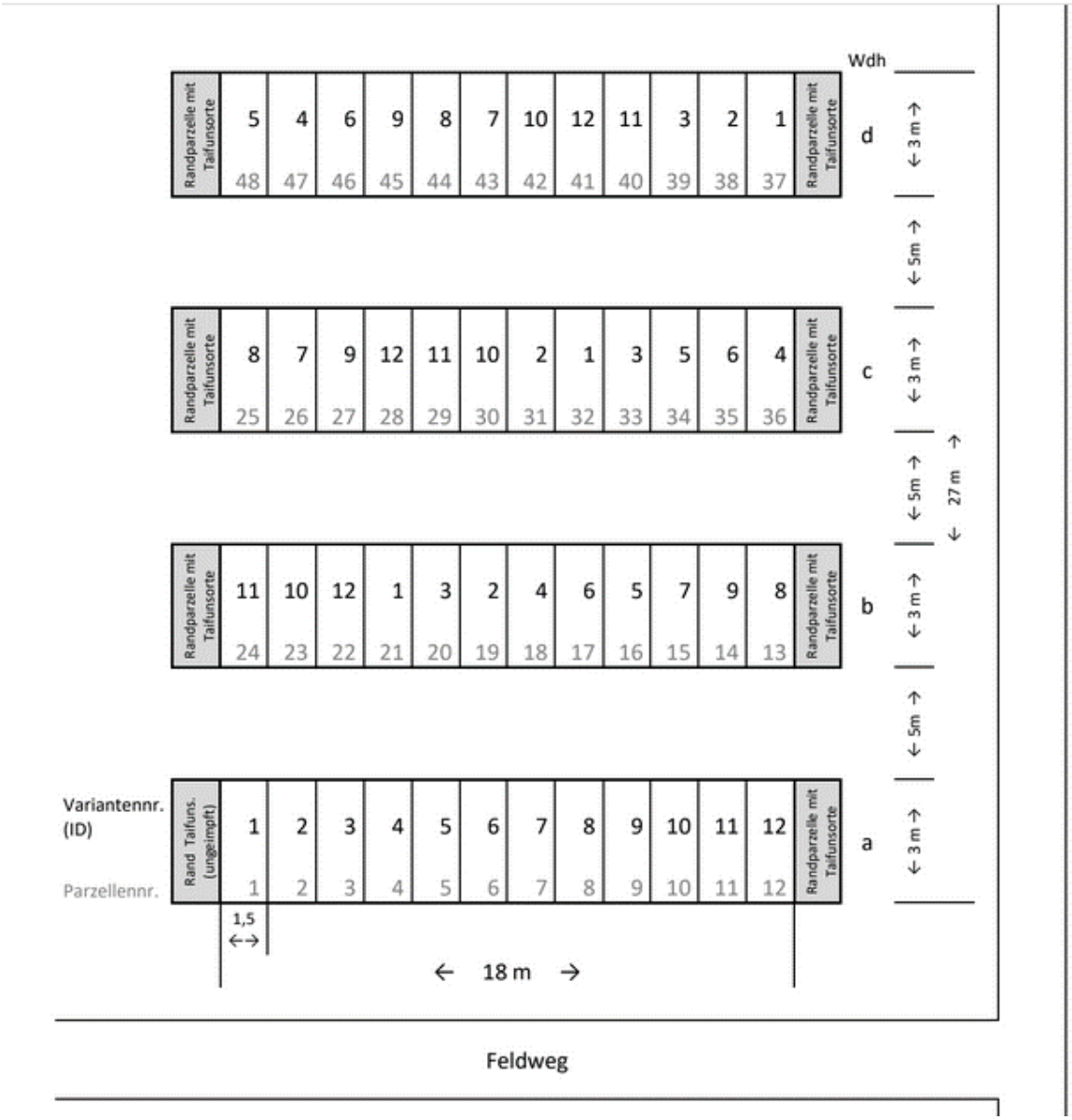

Figure 33 Site plan of the field trial in 2016

															Wdh			
Treatment	p	k	pm	p	k	m	p	pm	p	m	m	k	pm	k	D	3	← 3 m →	↓ 27 m ↓
Variety	M	P	P	E	E	P	P	E	M	E	M	M	M	M				
Parzellennr.	R8	148	147	146	145	144	143	142	141	140	139	138	137	R7				
																5	← → 5 m	
Treatment	k	pm	k	pm	k	p	k	m	m	p	m	p	pm	p	C	3	← 3 m →	
Variety	M	E	M	P	E	P	P	M	E	E	P	M	M	M				
Parzellennr.	R5	125	126	127	128	129	130	131	132	133	134	135	136	R6				
																5	← → 5 m	
Treatment	p	k	m	k	p	pm	p	p	pm	k	m	m	pm	k	B	3	← 3 m →	
Variety	M	E	M	P	P	P	M	E	M	M	E	P	E	M				
Parzellennr.	R4	124	123	122	121	120	119	118	117	116	115	114	113	R3				
																5	← → 5 m	
Treatment	k	m	pm	pm	m	p	m	p	k	p	k	k	pm	p	A	3	← 3 m →	
Variety	M	E	M	E	P	M	M	P	P	E	M	E	P	M				
Parzellennr.	R1	101	102	103	104	105	106	107	108	109	110	111	112	R2				
		1.5																
		← →																
	← 21 m →																	

Erläuterung:	
Variety	Treatment
E - ES Mentor	m - mykoplant
M - Merlin	p - priming
P - Primus	k - kontrolle
	pm - priming und mykoplant

Figure 34 Site plan of the field trial in 2017

Table 28 Mean values and SD for all traits taken in the field experiment

			treatment control		priming		Mykoplant®		priming and Mykoplant®	
days 09			14	± 6	15	± 6	14	± 6	15	± 6
days 10			17	± 5	17	± 5	17	± 5	17	± 5
days 11			19	± 5	20	± 4	20	± 5	20	± 4
days 12			29	± 7	29	± 7	29	± 7	30	± 7
emergence (%)	2016	ES Mentor	68.0	± 10.1	51.3	± 2.6	80.3	± 7.9	53.0	± 7.3
		Merlin	89.3	± 7.4	62.8	± 7.5	84.3	± 9.4	54.5	± 7.7
		Primus	86.3	± 1.7	68.0	± 7.0	86.0	± 3.9	59.0	± 8.8
	2017	ES Mentor	90.5	± 4.7	57.3	± 10.8	89.3	± 5.1	70.0	± 3.4
		Merlin	96.0	± 2.2	71.3	± 6.4	95.5	± 4.1	75.3	± 2.2
		Primus	72.8	± 7.4	33.8	± 9.8	71.0	± 11.5	30.0	± 3.4
yield (dt ha^{-1})	ES Mentor		51.9	± 9.0	52.6	± 7.0	55.3	± 7.8	54.7	± 9.8
	Merlin		44.4	± 5.6	44.3	± 7.7	41.7	± 2.7	41.1	± 7.3
	Primus		48.0	± 3.5	34.8	± 10.3	42.6	± 5.1	37.9	± 5.7
N Content (%) in DM	2016		7.40	± 0.44	7.33	± 0.34	7.37	± 0.42	7.41	± 0.37
	2017		7.38	± 0.54	7.50	± 0.46	7.53	± 0.51	7.44	± 0.51
TGW (g) dried			190.8	± 40.6	184.8	± 37.7	189.3	± 40.1	186.6	± 40.3
height 1. pod (cm)			13.9	± 2.7	13.3	± 3.0	14.1	± 3.1	13.0	± 3.4
height (cm) at BBCH 12	ES Mentor		12.0	± 1.5	11.6	± 1.2	12.1	± 1.8	11.8	± 1.6
	Merlin		16.1	± 1.0	14.1	± 1.3	15.8	± 1.2	13.9	± 1.5
	Primus		12.7	± 0.5	11.2	± 0.6	11.9	± 1.0	10.8	± 0.7
height (cm) at blossom			55.7	± 17.7	48.9	± 16.3	54.8	± 17.9	48.6	± 17.1
height (cm) 6 w after blossom in 2016			88.0	± 9.7	83.6	± 11.6	87.1	± 9.9	83.1	± 10.3
height (cm) at harvest			93.0	± 12.0	88.6	± 13.9	92.6	± 13.4	88.7	± 12.9
LAI at blossom in 2016			4.13	± 1.77	2.46	± 1.31	3.82	± 1.13	2.21	± 1.34
LAI 6 w after blossom in 2016			7.52	± 1.12	7.30	± 0.85	7.53	± 0.99	7.18	± 1.19
Chlorophyll at BBCH 12			519.3	± 41.3	519.0	± 36.0	516.4	± 39.9	515.0	± 39.7
Chlorophyll at blossom	2016		495.9	± 25.9	468.8	± 16.7	483.9	± 17.0	470.1	± 24.8
	2017		514.3	± 19.8	527.6	± 13.0	514.0	± 15.9	523.3	± 14.5

	treatment control		priming		Mykoplant®		priming and Mykoplant®	
Chlorophyll 6 w after blossom (2016)	621.7	± 16.2	617.0	± 18.7	617.8	± 19.1	615.2	± 19.0
shoot DM(mg) at BBCH 12 in 2017	839.0	± 132.1	770.8	± 169.5	851.0	± 128.7	743.5	± 72.0
shoot DM (g) at blossom in 2017	14.00	± 4.41	18.0	± 7.6	15.1	± 4.4	17.3	± 5.9
root DM (mg) at BBCH 12 in 2017	149.8	± 26.1	154.4	± 27.8	166.7	± 32.4	135.6	± 18.5
root DM (g) at blossom in 2017	2.1	± 0.5	2.6	± 0.6	2.4	± 0.5	2.6	± 0.6
nodules DM (mg) at blossom	180.5	± 52.1	166.3	± 62.5	170.7	± 69.1	173.5	± 36.9
no. of nodules at BBCH 12	17	± 9	16	± 8	17	± 7	17	± 8
no. of nodules at blossom	47	± 17	50	± 16	50	± 18	55	± 13

Table 29 ANOVA Table for the traits taken in the field experiment (without BBCH stages, they are separate). Data shown are the p-values

source of variation	emergence	yield	N Content (%)	TGW	no. of pods	height 1.pod	crop height at BBCH 12	crop height at blossom	crop height 6 w after blossom (2016)	crop height at harvest	LAI at blossom (2016)	LAI 6 w after blossom (2016)	Chlorophyll at BBCH 12	Chlorophyll at blossom	Chlorophyll 6 w after blossom (2016)
year	0.005	0.004	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	na	<0.001	na	na	0.84	<0.001	na
variety	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.009	<0.001	<0.001	0.005	<0.001
treatment	<0.001	0.043	0.432	0.009	<0.001	0.003	<0.001	<0.001	0.311	0.002	0.002	0.261	0.716	0.352	0.765
year-variety	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	na	0.275	na	na	<0.001	0.002	na
year-treatment	0.026	0.082	0.048	0.753	0.068	0.514	0.182	0.329	na	0.774	na	na	0.75	<0.001	na
variety-treatment	0.021	0.003	0.494	0.056	0.371	0.052	0.031	0.294	0.999	0.267	0.866	0.060	0.751	0.967	0.796
year-variety-treatment	0.03	0.492	0.263	0.199	0.108	0.652	0.747	0.876	na	0.459	na	na	0.942	0.284	na
levene (based on mean)	0.205	<0.001	0.003	0.045	0.048	0.03	0.452	0.024	<0.001	<0.001	0.305	0.019	0.451	0.153	0.653
shapiro-wilk	0.405	0.652	0.61	0.417	0.205	0.657	0.547	0.484	0.737	0.594	0.139	0.488	0.068	0.205	0.153

Table 29 (cont.) ANOVA Table for the traits taken in the field experiment (without BBCH stages, they are separate). Data shown are the p-values

source of variation	shoot DM at BBCH 12 in 2017	shoot DM at blossom in 2017	root DM at BBCH12 in 2017	root DM at blossom in 2017	nodules DM at blossom in 2017	no. of nodules at BBCH12	no. of nodules at blossom
year	na	na	na	na	na	<0.001	<0.001
variety	0.88	<0.001	0.171	<0.001	0.042	0.001	<0.001
treatment	0.193	0.102	0.06	0.053	0.929	0.745	0.143
year-variety	na	na	na	na	na	0.136	0.448
year-treatment	na	na	na	na	na	0.261	0.61
variety-treatment	0.936	0.144	0.792	0.611	0.393	0.678	0.208
year-variety-treatment	na	na	na	na	na	0.996	0.757
levene (based on mean)	0.003	0.136	0.022	0.107	0.617	0.13	0.079
shapiro-wilk	0.964	0.136	0.722	0.805	0.2	0.488	0.303

Table 30 Correlation matrix field traits

Pearson Correlation and sign. (2-tailed)		soil N	emergence (%)	Chlorophyll at BBCH 12	Chlorophyll at blossom	crop height at BBCH 12	crop height at blossom	crop height at harvest	no. of nodules at BBCH 12	no. of nodules at blossom	height 1.pod	no. pods	TGW	yield (dt ha^{-1})	N Content (%) in DM	days 09	days 10	days 11	days 12
soil N content	r	1.00	-0.07	-0.71	0.63	0.27	0.73	0.56	-0.72	-0.56	0.63	-0.27	0.44	0.07	0.06	-0.80	-0.81	-0.80	-0.83
	p		0.53	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.48	0.56	0.00	0.00	0.00	0.00
emergence (%)	r	-0.07	1.00	0.10	0.16	0.59	0.31	0.06	0.02	-0.20	0.37	-0.44	-0.24	0.41	-0.31	-0.22	-0.14	-0.20	-0.11
	p	0.53		0.34	0.13	0.00	0.00	0.56	0.87	0.05	0.00	0.00	0.02	0.00	0.00	0.03	0.16	0.05	0.29
Chlorophyll at BBCH 12	r	-0.71	0.10	1.00	-0.49	-0.33	-0.79	-0.64	0.77	0.55	-0.53	0.27	-0.35	0.19	-0.03	0.73	0.79	0.78	0.79
	p	0.00	0.34		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.06	0.74	0.00	0.00	0.00	0.00
Chlorophyll at blossom	r	0.63	0.16	-0.49	1.00	0.41	0.73	0.69	-0.54	-0.51	0.61	-0.40	0.42	0.12	0.13	-0.69	-0.70	-0.71	-0.73
	p	0.00	0.13	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.20	0.00	0.00	0.00	0.00
crop height at BBCH 12	r	0.27	0.59	-0.33	0.41	1.00	0.63	0.19	-0.34	-0.41	0.51	-0.17	-0.38	0.12	-0.49	-0.47	-0.40	-0.47	-0.39
	p	0.01	0.00	0.00	0.00		0.00	0.06	0.00	0.00	0.00	0.10	0.00	0.26	0.00	0.00	0.00	0.00	0.00
crop height at blossom	r	0.73	0.31	-0.79	0.73	0.63	1.00	0.76	-0.76	-0.67	0.81	-0.44	0.30	0.12	-0.06	-0.91	-0.91	-0.93	-0.90
	p	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.26	0.56	0.00	0.00	0.00	0.00
crop height at harvest	r	0.56	0.06	-0.64	0.69	0.19	0.76	1.00	-0.57	-0.53	0.50	-0.62	0.70	-0.09	0.50	-0.64	-0.71	-0.71	-0.71
	p	0.00	0.56	0.00	0.00	0.06	0.00		0.00	0.00	0.00	0.00	0.00	0.37	0.00	0.00	0.00	0.00	0.00
no. of nodules at BBCH 12	r	-0.72	0.02	0.77	-0.54	-0.34	-0.76	-0.57	1.00	0.68	-0.63	0.29	-0.33	0.07	0.02	0.78	0.81	0.80	0.80
	p	0.00	0.87	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.51	0.82	0.00	0.00	0.00	0.00
no. of nodules at blossom	r	-0.56	-0.20	0.55	-0.51	-0.41	-0.67	-0.53	0.68	1.00	-0.54	0.41	-0.25	-0.02	-0.04	0.63	0.64	0.65	0.63
	p	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.01	0.88	0.67	0.00	0.00	0.00	0.00
height 1.pod	r	0.63	0.37	-0.53	0.61	0.51	0.81	0.50	-0.63	-0.54	1.00	-0.36	0.22	0.38	-0.15	-0.88	-0.84	-0.83	-0.82
	p	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.03	0.00	0.13	0.00	0.00	0.00	0.00
no. of pods	r	-0.27	-0.44	0.27	-0.40	-0.17	-0.44	-0.62	0.29	0.41	-0.36	1.00	-0.46	0.09	-0.38	0.35	0.37	0.40	0.36
	p	0.01	0.00	0.01	0.00	0.10	0.00	0.00	0.00	0.00	0.00		0.00	0.38	0.00	0.00	0.00	0.00	0.00
TGW	r	0.44	-0.24	-0.35	0.42	-0.38	0.30	0.70	-0.33	-0.25	0.22	-0.46	1.00	0.07	0.81	-0.39	-0.47	-0.42	-0.50

Pearson Correlation and sign. (2-tailed)		soil N	emergence (%)	Chlorophyll at BBCH 12	Chlorophyll at blossom	crop height at BBCH 12	crop height at blossom	crop height at harvest	no. of nodules at BBCH 12	no. of nodules at blossom	height 1.pod	no. pods	TGW	yield (dt ha^{-1})	N Content (%) in DM	days 09	days 10	days 11	days 12
	p	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.00		0.52	0.00	0.00	0.00	0.00	0.00
yield	r	0.07	0.41	0.19	0.12	0.12	0.12	-0.09	0.07	-0.02	0.38	0.09	0.07	1.00	-0.13	-0.32	-0.25	-0.22	-0.24
(dt ha^{-1})	p	0.48	0.00	0.06	0.25	0.26	0.26	0.37	0.51	0.88	0.00	0.38	0.52		0.19	0.00	0.01	0.03	0.02
N Content	r	0.06	-0.31	-0.03	0.13	-0.49	-0.06	0.50	0.02	-0.04	-0.15	-0.38	0.81	-0.13	1.00	0.05	-0.05	0.01	-0.08
(%) in DM	p	0.56	0.00	0.74	0.20	0.00	0.56	0.00	0.82	0.67	0.13	0.00	0.00	0.19		0.65	0.65	0.93	0.46
days 09	r	-0.80	-0.22	0.73	-0.69	-0.47	-0.91	-0.64	0.78	0.63	-0.88	0.35	-0.39	-0.32	0.05	1.00	0.99	0.98	0.97
	p	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.65		0.00	0.00	0.00
days 10	r	-0.81	-0.14	0.79	-0.70	-0.40	-0.91	-0.71	0.81	0.64	-0.84	0.37	-0.47	-0.25	-0.05	0.99	1.00	0.98	0.98
	p	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.65	0.00		0.00	0.00
days 11	r	-0.80	-0.20	0.78	-0.71	-0.47	-0.93	-0.71	0.80	0.65	-0.83	0.40	-0.42	-0.22	0.01	0.98	0.98	1.00	0.97
	p	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.93	0.00	0.00		0.00
days 12	r	-0.83	-0.11	0.79	-0.73	-0.39	-0.90	-0.71	0.80	0.63	-0.82	0.36	-0.50	-0.24	-0.08	0.97	0.98	0.97	1.00
	p	0.00	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.46	0.00	0.00	0.00	

8.4 Literature overview of papers reporting about hydropriming in soybean

Table 31 Literature overview of studies showing positive effects of priming on final germination

Reference	Hydro in title	Country	Initial germ (%)	Temp germ (°C)	medium	Effect of priming	soybean variety	Priming description
Bharati et al. (1983)	yes	Australia	92.5	10	paper	MTE is reduced, germination increased	Gilbert (116), Semstar (138), P 12 (207), P33 (184)	32 h priming in distilled water at 31°C (w/o and with drying)
Arif et al. (2008)	no	Pakistan		field	field	emergence increased from 66 to 73 after 6 h under high temp field conditions (soil temp > 20°C)	William 82 (MG3)	6h, 12, 18h of priming in deionized water dried to 8% moisture
Mohammadi (2009)	no	Iran	57	5, 15, 25, 35	paper	Germination increased to 63. Fester emergence and higher yields	Williams (MG3)	24 h at 20°C distilled water, than surface dried
Bejandi et al. (2009)	no	Iran	65	25		Germination increased to 86%, faster emergence	Williams (MG3)	aerated water 12h at 25°C
Maroufi and Farahani (2011)	yes	Iran	60	25	paper	Germination increased to 73%. And plants had more biomass when seeds were primed	na	12 and 24 h
Moosavi et al. (2012)	yes	Iran	41.85	field	field	54% after 8 h, the longer the lower (31% after 20 h). Plants grow taller	Williams and LV 17	8, 12, 15, 20 h in tap water at 17.3°C, dried to 30%moisture
Miladinov et al. (2014)	no	Serbia	76	field	field	Soaking the seed in distilled water also leads to improvement of soybean seed germination and vigour, but less than when soaking it in other primers.	old variety	6 h soaked in and dried at 25 h to initial moisture content
Moosavi et al. (2014)	yes	Iran	87	10	paper	higher germination: 98, 93 after 8 and 12 h (16 and 20 h led to a reduction), 8h led to higher yield	Williams + LV17	8, 12, 16 and 20 h in distilled water at 17.3°C and dried to 30% moisture at 20 to 22°C

Reference	Hydro in title	Country	Initial germ (%)	Temp germ (°C)	medium	Effect of priming	soybean variety	Priming description
Arif et al. (2014)	no	Pakistan	47.3	25	silt	increased to 57% after 6 h, decreasing to 44% after 18 h	William 82 (MG3)	6, 12, 18 h, different PEG concentrations and water aerated with an aquarium pump
Iqbal et al. (2015)	no	Pakistan	58	Outside?	pot	final emergence higher and start of emergence faster	Ajmiri	12 h in distilled water or tap water
Kujur and Lal (2015)	yes	India	65	25	paper	67,69, 71, 69 after 8, 12, 24 and 48 h. mean germination time did not increased, DM and length of plants increased when primed	DS-2706	8, 12, 24 and 48 h at 25°C between wet towel papers (-> solid matrix priming)
Langeroodi and Noora (2017)	no	Iran	85	25 and field	paper and field	higher and faster germination and yield increase	Williams, DPX	12 and 18 h in distilled water, dried to original moisture content
Mohamed et al. (2018)	no	Egypt	40	25	ISTA	germination increased to 51%	Giza 111	6, 12, and 24 h between towels (matrix priming), dried to initial moisture content (10%)

Table 32 Literature overview of studies showing negative effects of priming on final germination

Reference	Hydro in title	Country	Initial germ (%)	Temp germ (°C)	medium	Effect of priming	soybean variety	Priming description
Helsel et al. (1986)	no	USA	95	~ 10	field	Reduction of initial and final emergence. Lowest yield with hydropriming	McCall (MG0), SRF 250 (MG2), Asgrow A3127 and Cumberland and Fayette and Williams 79 and Williams 82 (MG3), Fortune 727 and Desoto and Franlin (MG4), Essex (MG5	4 h soaked in distilled water, air dried and planted after 4 h
Ghassemi-Golezani et al. (2011)	no	Iran	97	15 and field	paper and field	Germination and Emergence reduced to about 25-40%. No faster germination or emergence	Zan	8 h ta 15°C, dried to 30-40% moisture
Sibande et al. (2015)	no	Malawi	90	room temp	lab	rate of germination and germination % are reduced	Serenade	6 h soaked, dried 48 h
Kering and Zhang (2015)	no	USA	63.5	field	field	varieties showed reduced emergence (or no effect)	MFS-561 (100g), V08-4773 (100g), Glenn (140g), V03-4705 (140g), MFL-159 (200g), V07-1897 (200g)	5 and 10 h soaking in water, different varieties were tested
Miladinov et al. (2018a)	no	Serbia	64	25	field	Hydropriming showed no significant effect or a reduction in germination. Old seeds with low initial germination showed sign reduction when hydroprimed	MG0: Galina, NS Princeza MG1: Sava, NS Apolo MG2: Rubin, NS Zita	6 h in distilled water, dried to 11% moisture content
Weerasekara et al. (2021)	no	Malaysia	59	26	sand	Germination percentage decreased, faster emergence with hydroprimed seeds. No sign interaction of soybean variety and priming duration	PB-1	1, 3, 5 and 7 h at 25°C in distilled water
Aminu et al. (2022)	yes	Nigeria	na	na	field	lower stand count	TGX-1835, TGX-1904, TGX-1951 and TGX-1955	4, 6, 8 h in tap water, dried superficially

Table 33 Literature overview of studies showing no or minor effects of priming on germination

Reference	Hydro in title	Country	Initial germ (%)	Temp germ (°C)	medium	Effect of priming	soybean variety	Priming description
Assefa and Hunja (2010)	no	India	97.5	25 and field	ISTA (Sand or paper), field	faster emergence	na	14 h between moist germination paper (solid matrix) and surface dried
Rouhi et al. (2011)	no	Iran	55-80	25	paper	speed of germination was decreased	Sari	12h at 25°C in distilled water
Costa et al. (2013)	yes	Brazil	94	25 and green-house	paper and sand	No change in germination or emergence. But seeds with fungal incidence hydropriming reduced germination and emergence about 20%	M-SOY 7908 RR	48 h at 20°C between wet paper (solid matrix), dried back (at 30°C) to 10% moisture
Moshtaghi-Khavaran et al. (2014)	no	Iran	88	15	paper	in aged seed, germ was initially lower and could be increased by halo priming, not hydropriming	L17	12 h in distilled water

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Erklärung

gemäß der Promotionsordnung des Fachbereichs 09 vom 07. Juli 2004 § 17 (2)

Ich erkläre: „Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe.

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