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THE EFFECTIVENESS OF SYNTHETIC POLYMERS IN REDUCING IMBIBITIONAL CHILLING STRESS IN GERMINATING BEAN SEEDS

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Abstract. The effectiveness of hydrophobic synthetic polymers in reducing imbibitional chilling stress in germinating bean seeds was evaluated. Two technical polymers and three Polish snap bean cultivars were analysed. Polymers were applied in amounts corresponding to 3.5% of seed weight. In the imbibitional chilling tolerance test, the seeds were rolled up in wet filter paper and left at 5°C for 72 hours, after which they were germinated at 20°C. The seed imbibition rate, germination energy, germination capacity and mean germination time were determined. The polymer coatings applied formed a barrier which slowed down seed imbibition, but it also significantly reduced germination energy and led to a decrease in germination capacity. The extent to which imbibitional chilling stress was mitigated was determined by the type of the polymer applied and the bean genotype analysed.

Keywords: film coating, germination, imbibitional chilling stress, snap bean seeds

INTRODUCTION

Film coating involves the placement of a thin layer of natural or synthetic polymer on seed surface. Atomised polymer droplets are deposited on the surface of diasporas to form a membranous coating (Taylor *et al*. 1998). Film coating is a seed enhancement technique which supports precision seeding by reducing seed friction, improving seed movement in seeding units and decreasing dust emissions. Polymers can be combined with dyes for seed labelling. They are most often used for the application of seed treatments because even very small amounts of active substances can be evenly distributed across the entire seed surface (Halmer 2006). New polymer technologies supply compounds which have different hydrophobic properties, are permeable to water and oxygen and are capable of releasing active substances under specific environmental conditions, over different periods of time and to a varied degree (Halmer 2008).

Bean seeds are large, they have a high content of hydrophilic substances and they quickly imbibe water during germination. When dry seeds with a very low water potential are placed in wet soil, they rapidly absorb water, which can lead to imbibitional injury (Prusiński 1997). The resulting damage is much more extensive when the absorbed water has a low temperature, and this phenomenon is known as imbibitional chilling stress (Knypl 1983). Cold water stress during imbibition decreases the field emergence capacity, increases the number of abnormal seedlings with cracked cotyledons and deformed roots, delays growth and decreases yield (Wolk and Herner 1982). Chilling damage can be mitigated by increasing the moisture content of sown seeds to slow down imbibition, but this practice can accelerate the aging of stored seeds (Prusiński 1990). The morphological parameters of the seed coat also affect the imbibition rate. Semi-hard seeds of selected bean genotypes have a semi-permeable layer which slows down water absorption during sprouting (Taylor and Kwiatkowski 2000). Film coating can be applied also to decrease the rate of seed hydration (Gesch *et al*. 2012).

This study evaluates the effect of seed coating with synthetic technical polymers on the germination of three snap bean (*Phaseolus vulgaris* L.) genotypes exposed to imbibitional chilling stress.

MATERIALS AND METHODS

Three snap bean (*Phaseolus vulgaris* L.) genotypes were used in the experiment: Paulinera (P), Uniwersa (U) ("Spójnia" – Hodowla i Nasiennictwo sp. z o.o. in Nochowo) and Jagusia (J) (Torseed – Przedsiębiorstwo Nasiennictwa Ogrodniczego i Szkółkarstwa S.A. in Toruń). Paulinera and Uniwersa are small seeded cultivars with a white seed coat, whereas Jagusia has large, dark red coloured seeds.

Seeds were coated with two types of synthetic technical polymers supplied by Henkel (H) and Akzo Nobel (ICI), using a laboratory atomiser. Polymer H is an aqueous dispersion of synthetic resins based on cellulose propionate and styrene, whereas polymer ICI is an acrylic copolymer emulsion. Warm air was supplied during the coating process to speed up film drying. The polymers were applied in amounts corresponding to 3.5% of seed weight.

The moisture content of coated seeds was adjusted to 12% in closed plastic containers over a water-glycerol solution (Taylor 1997). The solution density was regularly monitored to maintain the required relative air humidity.

The imbibition rate of coated and control seeds was determined at 20°C. Three groups of 10 seeds from every cultivar and coating treatment were weighed on a WM118 laboratory scale (MeraMont) with the precision of 0.005 g. The seeds were placed in 50 ml flasks filled with distilled water. The seeds were weighed every 30 minutes in the first 4 hours of immersion, every hour during the successive 8 hours of imbibition, and the last measurement was performed after 24 hours of immersion. Before every measurement, seeds were strained in a sieve and surface dried on a paper towel.

In the imbibitional chilling tolerance test, the seeds were rolled up in wet filter paper and left at 5°C for 72 hours. The test was carried out in 4 replications of 25 seeds each for every cultivar and coating treatment. Rolled up seeds were placed in flasks containing several centimetres of water cooled to 5°C to saturate filter paper and the seeds. The flasks were covered with plastic bags to maintain constant relative humidity of air surrounding the seeds. After 72 hours of saturation, the flasks were stored at 20°C for 9 days, and germination parameters were determined (Sanyo MLR-350T Plant Growth Chamber). Stress conditions were selected based on a preliminary experiment which evaluated the germination parameters of seeds stored in wet filter paper at 5°C for 24, 48 and 72 hours or at 10°C for 48, 96 and 192 hours. Imbibitional chilling damage was intensified in the cold stress regime selected. Seeds wrapped in wet filter paper without exposure to cold stress and germinated at 20°C were the control.

The initial ("germination energy") and final ("germination capacity") counting of normal seedlings was performed after 5 and 9 days of germination under optimal conditions (20°C), respectively, in accordance with the methodology proposed by ISTA Rules for Seed Testing (Anonymous 2010).

Pieper's coefficient, an indicator of the mean time of germination of a single seed, was determined in additional replications for every tested combination (Pieper 1952). The number of newly germinated seeds was counted every day at the same time, and Pieper's coefficient was determined with the use of the below formula:

$$E = \frac{n_1 S_1 + n_2 S_2 + ... n_m S_m}{n_1 + n_2 + ... n_m}$$

where: E – mean germination time; n – counting day; S – number of seeds germinated on a given day; m – last day of measurement. The resulting coefficients were converted to hours.

The results were processed in the Statistica® application.

RESULTS AND DISCUSSION

Seed imbibition

Imbibition curves for raw seeds of the tested bean cultivars illustrate the first two stages of the germination process (Fig. 1). The first stage lasted from 2 to 6 hours, subject to cultivar, and it was characterised by high water absorption rates. The highest imbibition rate was observed in Paulinera seeds, but Uniwersa seeds absorbed the largest amount of water relative to their initial weight (around 130%). The second stage was terminated after 24 hours of imbibition, and it was characterised by low water absorption rates.

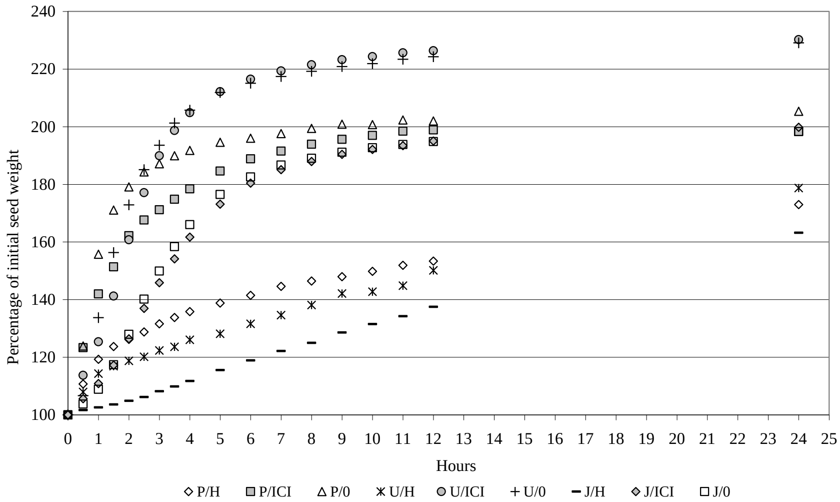


Fig. 1. Imbibition curves of raw and coated bean seeds at a temperature of 20°C. (Explanations are given in the Materials and Methods section)

Film coating led to changes in imbibition rates. Polymer ICI insignificantly slowed down seed hydration in the first stage of the process, but the differences noted were levelled out after 4-12 hours of imbibition, subject to cultivar. Polymer H significantly limited the seeds' access to water, and it radically decreased the rate and degree of seed hydration. After 24 hours of imbibition, the moisture content of seeds coated with polymer H was reduced to the level of 78-85% of raw seeds.

Seeding value of analysed seeds

Raw seeds were characterised by high seeding value, and the percentage of sprouting seeds in the first counting (Fig. 2) conformed to the minimum certification requirements set forth by the Regulation of the Polish Minister of Agriculture and Rural Development (Anonymous 2007). Germination capacity was similar to germination energy, and it was 4% higher only in cv. Jagusia (Fig. 3). The mean germination time was 52 hours (Fig. 4). The shortest germination time was reported in Uniwersa seeds, and it corresponded with the imbibition rate for that cultivar (Fig. 4).

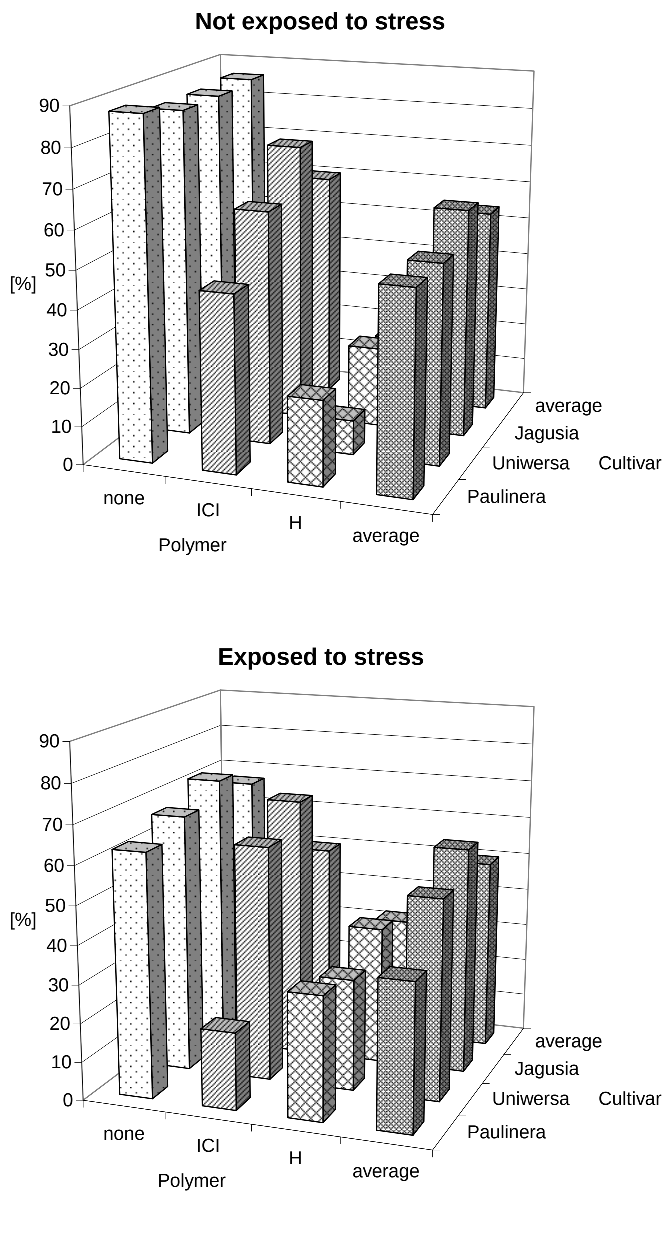
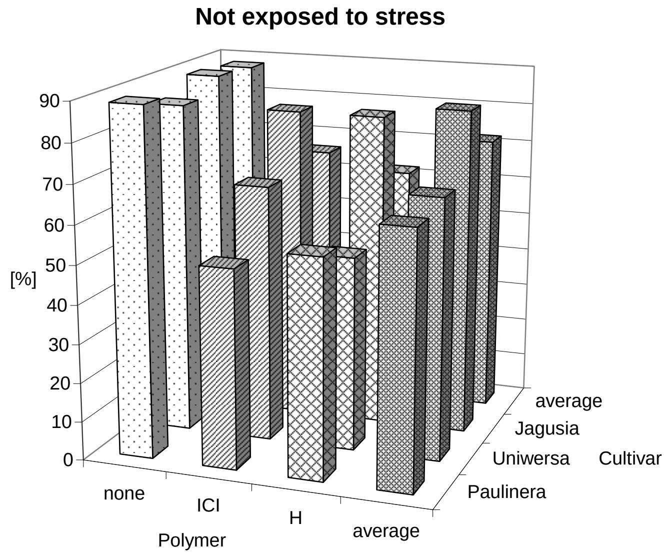


Fig. 2. Germination energy (%) of polymer-coated seeds not exposed and exposed to imbibitional chilling stress



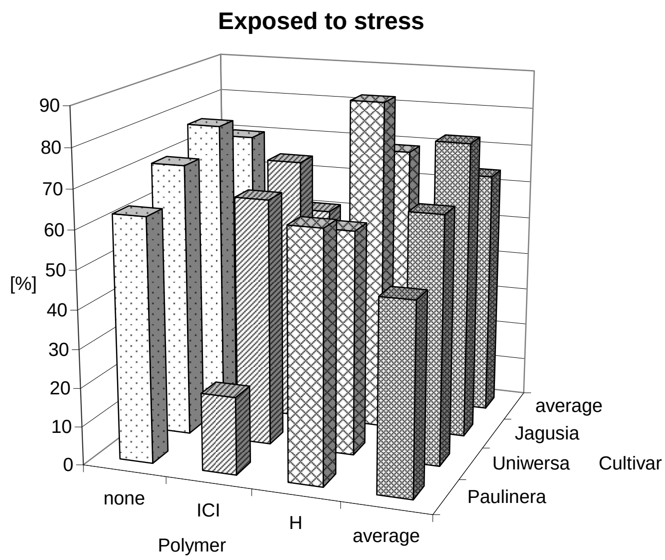
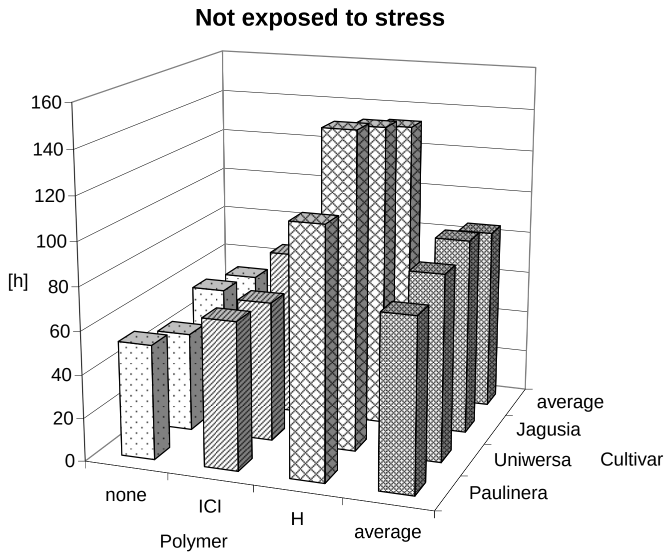


Fig. 3. Germination capacity (%) of polymer-coated seeds not exposed and exposed to imbibitional chilling stress



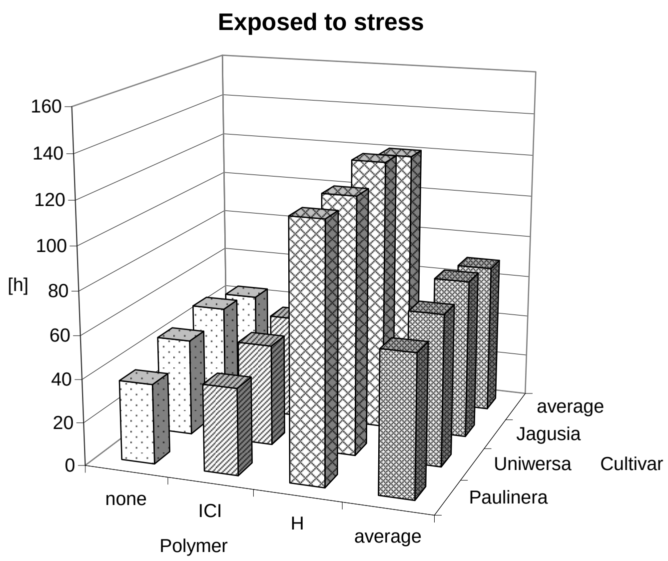


Fig. 4. Mean germination time (h) of polymer-coated seeds not exposed and exposed to imbibitional chilling stress

Response to imbibitional chilling stress

Imbibitional chilling stress reduced the number of normally sprouted seeds by 19% on average during the first counting (Fig. 2). The greatest differences were observed in var. Paulinera which is characterised by the highest germination capacity under optimal conditions. Least significant variations were reported for Jagusia seeds.

The exposure of germinating seeds to imbibitional chilling stress decreased the number of normal seedlings by 18% on average during the final counting (Fig. 3). The sprouting of all of the analysed bean cultivars was delayed in comparison with the control. Jagusia was most resistant to imbibitional chilling stress: its germination capacity reached 77% and it was only 12% lower than that observed in an optimal growing environment. The above cultivar was highly tolerant to imbibition at low temperature, and its germination capacity values remained above the threshold set by the applicable standards. The highest reduction in the number of normal seedlings (25%) in the final counting was reported for Paulinera which was most sensitive to cold water stress during imbibition.

The mean germination time was 44 hours, and imbibitional chilling stress decreased the above parameter by 8 hours in comparison with the control (Fig. 4). Paulinera seeds were characterised by the shortest germination time and Pieper's coefficient of 37 h which was 16% lower than in the control. Jagusia seeds were characterised by the highest seeding value under exposure to imbibitional chilling stress, and the longest mean germination time.

Effect of film coating on the seeding value of bean seeds

Seed coating with hydrophobic technical polymers significantly lowered the germination energy after 5 days of sprouting under optimal conditions. Polymer ICI reduced the germination capacity by 26%, and polymer H – by 69% (Fig. 2). Jagusia seeds were least sensitive to film coating which induced a 12% decrease in the number of normal seedlings during the initial measurement. The greatest drop in germination viability was noted in Paulinera seeds. Polymer H inhibited germination in all three bean cultivars. The smallest reduction was reported in Paulinera and Jagusia, and the greatest – in Uniwersa seeds where the application of polymer H decreased the number of normal seedlings 9-fold in the first measurement.

The percentage share of normal seedlings produced by coated seeds after 9 days of germination was 22-26% lower on average in comparison with raw seeds (Fig. 3). The effect of film coating on seed germination under optimal conditions differed subject to cultivar, and the smallest reduction (8%) was noted in Jagusia seeds for both polymers. Film coating significantly lowered the seeding value of cv. Paulinera and Uniwersa. The germination capacity of Uniwersa seeds coated with polymer ICI decreased by 19% and with polymer H – by 35% in comparison with the control. In Paulinera seeds, the application of polymer ICI led to a greater reduction in germination capacity. Coating with both tested polymers decreased the germination capacity of Paulinera and Uniwersa seeds below the minimum seed certification standards.

The results of germination analysis indicate that the decrease in the percentage of normal seedlings was caused by a more than three-fold increase in the number of dead seeds after coating with both tested polymers (Tab. 1).

Table 1. Additional parameters of germination test

Evaluated parameter	Polymer			Average	$LSD_{0.05}$
	None	ICI	H		
Dead seeds	8	25	25	19	6.9
Fresh seeds	1	5	10	5	4.3
Abnormally germinating seeds	3	4	3	3	–
Average	4	11	13	9	–

Polymer coating, in particular the application of polymer H, significantly decreased germination rates in comparison with the control (Fig. 4). The mean germination time of seeds coated with polymer H was 133 hours, and it was more than 2.5-times longer than that of control seeds. The above indicates that polymer H is a strongly hydrophobic substance which significantly slows down the rate of water absorption in seeds and decreases their germination rate. Polymer H had a particularly inhibiting effect on cv. Uniwersa which was characterised by the highest Pieper's coefficient of 146 h, i.e. more than three times higher than in control seeds. In seeds coated with polymer H, the lowest value of Pieper's coefficient at 114 h was noted in cv. Paulinera. Polymer ICI also inhibited water absorption and increased the value of Pieper's coefficient, but the variations observed were not significant. The most notable differences between seeds coated with polymer ICI and control seeds were observed in cv. Jagusia (21%), and the least significant differences – in cv. Paulinera (15%).

Effect of film coating on the germination of seeds exposed to imbibitional chilling stress

Film coating lowered the average germination energy of seeds exposed to imbibitional chilling stress in comparison with the control (Fig. 2). The reported decrease was more profound in seeds coated with polymer H than polymer ICI, and it was also determined by seed genotype. In the group of seeds coated with polymer ICI, the germination of Jagusia and Uniwersa seeds was only insignificantly lower than that of control seeds in the first counting, whereas more than a three-fold decrease was noted in the germination energy of Paulinera seeds. The application of polymer H reduced the germination energy by 50% in all three seed cultivars under exposure to imbibitional chilling stress.

The germination capacity of film-coated bean seeds exposed to imbibitional chilling stress varied subject to genotype (Fig. 3). The application of polymer H increased the germination capacity of cold-stressed Jagusia seeds by 9% in comparison with the control. The germination capacity of Paulinera seeds did not change in the analysed stress regime, whereas a 13% drop was observed in cv. Uniwersa. Polymer ICI insignificantly decreased the germination capacity of Jagusia and Uniwersa seeds (8-7%), but it induced more than a three-fold drop in the germination capacity of Paulinera seeds.

The application of polymer ICI did not significantly change the germination time of seeds exposed to imbibitional chilling stress in comparison with the control (Fig. 4). Polymer H induced an average 2.8-fold increase in mean germination time relative to the control. The above changes were observed in all analysed bean cultivars.

Film coating is a popular seed enhancement technique. New treatment methods rely on the functional properties of polymers which create a barrier that delays imbibition or enables seeds to absorb water within a given temperature range (Gesch *et al.* 2012). Polymers improve the field emergence of plants sensitive to imbibitional chilling stress, they are used to synchronise flowering in hybrid seed production or fall plantings of spring cultivars (Halmer 2006).

Taylor and Kwiatkowski (2001) and Ni (2001) demonstrated that seed coating with starch-based polymers enhances seed germination under exposure to imbibitional chilling stress and improves the emergence capacity of bean, corn and soybean genotypes sensitive to unsupportive growing conditions.

Hydrophobic polymers evaluated in this study validated the research hypothesis only partially. The polymers slowed down the rate of water absorption in the first stage of germination, but they failed to eliminate the negative effects of imbibitional chilling stress on bean seeds. The analysed polymers created a hydrophobic physical barrier which could have inhibited gas diffusion or exerted a toxic effect on germinating seeds, which was a common weakness of early seed coating techniques (Hill 1999). The polymer formulas applied were not designed specifically for seed coating. According to Christian *et al*. (2004), the use of polymers as barriers that slow down water absorption and minimise imbibitional chilling damage can decrease the field emergence capacity in water-deficient soils, in particular under exposure to low temperatures.

CONCLUSIONS

1. The analysed seeds were characterised by a high seeding value which significantly exceeded the minimum certification requirements for the evaluated cultivars.

2. Imbibitional chilling stress significantly lowered seed germination parameters, but the effects observed varied subject to genotype.

3. Seed coating with hydrophobic technical polymers slowed down imbibition rates, mainly in seeds coated with polymer H, reduced the germination energy, led to a decrease in the germination capacity and prolonged the mean germination time.

4. The extent to which imbibitional chilling stress was mitigated was determined by the type of the polymer applied and the bean genotype analysed. Positive effects of the treatment applied were noted only in bean seeds cv. Jagusia coated with polymer H.

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OCENA SKUTECZNOŚCI SYNTETYCZNYCH POLIMERÓW W OGRANICZENIU STRESU CHŁODNOWODNEGO PODCZAS KIEŁKOWANIA NASION FASOLI

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Streszczenie. W pracy oceniano skuteczność syntetycznych polimerów hydrofobowych w ograniczeniu stresu chłodnowodnego podczas kiełkowania nasion fasoli. Do badań wykorzystano dwa polimery techniczne oraz trzy odmiany fasoli szparagowej polskiej hodowli. Polimer naniesiono w ilości 3,5% masy nasion. Test chłodno-wodny przeprowadzono umieszczając nasiona w rulonach z mokrej bibuły w temperaturze 5°C na okres 72 godzin po czym prowadzono standardowe kiełkowanie w temperaturze 20°C. Oceniono tempo pęcznienia nasion, energię i zdolność kiełkowania oraz średni czas kiełkowania nasion. Stwierdzono, że naniesiona powłoka polimerowa stworzyła barierę spowalniającą tempo pęcznienia nasion ale równocześnie powodującą silną redukcję energii kiełkowania i częściowe obniżenie zdolności kiełkowania. Ograniczenie skutków stresu chłodnowodnego uzależnione było od zastosowanego polimeru i badanego genotypu.

Słowa kluczowe: cienkowarstwowe powlekanie, kiełkowanie, nasiona fasoli, stres chłodno-wodny