Influence of seed size and water restriction on germination of soybean seeds and on early development of seedlings[1]

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ABSTRACT – Germination is a biological process that depends on adequate water supply to embryo development. Water deficit slows this process and depending on intensity and extent of this deficit may cause seed death. Nevertheless, it has not yet been reported whether seed size influences physiological potential, or tolerance to water stress. This study aimed at assessing the effects of seed size, as well as of water stress on germination of the seeds and on early soybean seedling development. The experiment was composed by seeds of 10 soybean cultivars classified by metallic screens with three sizes of oval holes (S12, S13 and S14) and subjected to three water potentials (0, -0.1, and -0.2 MPa), with four replications. Data on genotypes were grouped as replications and arranged on a factorial 3 x 3 (size x water potential), with 40 replications. Data assessed were: first and final count of germination; length and seedling dry weight; and correlation between length/mass of radicle and hypocotyl. It was concluded that under ideal moisture conditions larger seeds have better physiological quality, producing more vigorous seedlings; but, that under water potential of -0.2 MPa smaller seeds produce larger seedlings; and that the hypocotyl is more influenced by water stress than the radicle.

Index terms: *Glycine max*, seed, water potential, germination.

Influência do tamanho de semente e da restrição hídrica na germinação de sementes de soja e no desenvolvimento inicial das plântulas

RESUMO – A germinação é um processo biológico dependente do fornecimento adequado de água para o desenvolvimento do embrião. O déficit hídrico retarda este processo e, dependendo da densidade e duração desse déficit, pode causar a morte da semente. Contudo, ainda não foi relatado se o tamanho da semente influencia o seu potencial fisiológico e a sua tolerância ao estresse. O objetivo deste estudo foi investigar os efeitos do tamanho da semente e do estresse hídrico na germinação das sementes e no desenvolvimento inicial das plântulas de soja. Este experimento foi composto por sementes de 10 cultivares de soja, classificadas por peneiras com crivos de três tamanhos (P12, P13 e P14) e submetidas a três potenciais hídricos (0, -0,1 e -0,2 MPa), com quatro repetições. Os dados sobre genótipos foram agrupados como repetições de um fatorial simples 3 x 3 (tamanhos x potenciais hídricos), com 40 repetições. Avaliou-se: primeira contagem e contagem final de germinação, e relação comprimento/massa do hipocótilo e da radícula. Concluiu-se que sob condições ideais de umidade, sementes maiores têm melhor qualidade fisiológica, produzindo plântulas mais vigorosas; mas, sob potencial hídrico de -0,2 MPa sementes menores produzem plântulas maiores; e que o hipocótilo é mais afetado pelo estresse hídrico do que a radícula.

Termos para indexação: *Glycine max*, semente, potencial hídrico, germinação.

Introduction

Water uptake by the seeds follow a pattern comprising three distinct phases: in the first phase (or imbibition phase) water is quickly absorbed, due to differences of water potential between substrate and seeds; in the sequence, second phase is characterized by a reduction on water absorption rate, which is the result of balance between the two potentials; and finally, in the third phase, due to metabolic activation and bioproduction of osmotically active substances by the seed, water returns

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to be rapidly absorbed (Bewley and Black, 1994). Therefore, germination is a biological process that is dependent on an adequate water provision for that the embryo development is resumed (Beckert et al., 2000).

If lack of water retards germination of the seeds and development of the seedlings, what may even lead them to death (Silva et al., 2006), and depending on intensity and/or extent of such water restriction (Moraes and Menezes, 2003), the fast imbibition caused by excess moisture available in environment, may result in damage to membranes of the seeds, thereby increasing rate of damages caused by over-imbibition (Silva and Villela, 2011).

In this sense, the irregularity in water availability during seed gemination is a relevant concern of producers, since it may limits the expression of productive potential of cultivars used, once periods of drought or excessive rains may occur during the seeding development. However, it should be emphasized that, in addition to environmental conditions, germination and the emergence of the seedlings also depend of the seed physiological potential (Moraes and Menezes, 2003).

Nevertheless, despite several studies on the subject, there is still no conclusive evidence that sorting of the seeds by size influence their physiological quality (Soltani et al., 2002); once uniformity in size, jointly with physical purity, genetic potential, high germination, lack of damages, and good health quality are the factors that actually indicate the good physiological quality of seeds (Jauer et al., 2002).

Costa et al. (2004) concluded that seed size does not affect their physiological quality, while Padua et al. (2010) obtained an exactly opposite conclusion. According to the results obtained in this last study, larger seeds present higher germination and vigor, produce taller plants, which have higher productivity at harvest. Moreover, standardization by seed size is relevant to accuracy of mechanical sowing (Krzyzanowski et al., 1991), as well as to improve visual and commercial aspect of the seed lots (Lima and Carmona, 1999).

Farmers often correlate the amount of reserves contained in the seed with a better performance on productivity in the field (Lima and Carmona, 1999). Nevertheless, Avila et al. (2008) verified that seed size affected neither weight of 100 seeds nor the yield of two soybean cultivars. Wheat seedlings originating from larger seeds have greater root length and a bigger hypocotyl (Soltani et al., 2002). However, Sung (1992) has found that the small seeds germinate faster, and produce plants with more developed root system, possibly due to lower water requirements for germination process. Later, Costa et al. (2004) reported that smaller seeds have higher germination percentage than the larger seeds under water stress above -1.0 MPa. On common bean crop, the use of large seeds may stimulate the early development of the crop (Perin et al., 2002), which may become advantageous for crop establishment in field under stress conditions (Lima et al., 2005). Within this context, Jauer et al. (2002) assessed four common bean cultivars and have verified that in one of them the smaller seeds presented low vigor, and that for the other cultivars there was no effect of seed size.

Seeds with high vigor generally provide higher productivity than the low vigor seeds (Scheeren et al., 2010), however the question if size of seeds has or has not effect on the seed physiological quality still has no definitive answer. Once there is much interest in clarifying this question, and if a correlation actually exists between size and physiological quality of the seeds, and if this correlation is maintained under adverse conditions, then new strategies may be studied aiming at improving establishment of the crop in areas where water deficit is common.

In face of the foregoing, regardless of genotype, this study aimed at assessing the effect of seed standardization by size and the water restriction on germination and early soybean seedlings development.

Material and Methods

The experiments were performed in the Seed Testing Laboratory of Department of Plant Science, Federal University of Viçosa, State of Minas Gerais, Brazil. The treatments were composed by 10 different soybean cultivars, which were arranged in a 3 x 3 factorial (3 seed sizes x 3 water potentials). For this, seed samples of the 10 genotypes used were taken at random from the following commercial soybean cultivars: CD 211; CD 217; CD 219RR; Elite; Confiança; Valiosa; UFVTN 105; Emgopa 315; Monarka; and UFV 16. The sorting was based on seed thickness and was performed using metal sieves (S), with oblong mesh, number: 12 (12 / 64 x ¾”), 13 (13/64 x ¾”); and 14 (14 / 64 x ¾”), hereinafter referenced in this text as S12, S13, and S14. Water restriction was simulated by using polyethylene glycol 6000 (PEG 6000), based on the scale developed by Villela et al. (1991), which simulates the water potentials of -0.1 and -0.2 MPa (Costa et al., 2012). Furthermore, it was used a control where the water restriction was simulated with distilled water. After sorting, seeds were subjected to the following tests:

Germination: this test was performed with four replicates of 50 seeds each, which were placed to germinate upon two sheets of Germitest® paper, covered with a third sheet, all of them moistened with water distilled or with PEG 6000 solution at -0.1 and -0.2 MPa in the ratio of 2.5 times the mass of the dry paper. The set (paper + seeds) was then turned into rolls that soon after were kept in a germination chamber (25 ± 1 °C) for incubation. First count of germination was performed on the fifth day after seeding; and final count of germination was made on the seventh day (Brasil, 2009).

Seedling length: was performed using the same methodology described for the germination test; however with four replicates and only 20 seeds per replication. Seeds were placed at the upper third of Germitest® paper, with the micropyle directed to paper base; thus favoring growth of seedlings (Nakagawa, 1999; Vanzolini et al., 2007). Seven days after seeding, the hypocotyl, the radicle, and the total height of seedlings were measured with the aid of a ruler graduated in centimeters. For obtaining the mean length of the hypocotyl, the mean length of radicle, and of the mean total length of the seedling, the assessments of each replication followed two different methodologies: 1) the sum of the total lengths of the seedlings divided by the number of seeds germinated (20) according to the International Seed Testing Association - ISTA (Hampton and TeKrony, 1995); and 2) the sum of the lengths of the hypocotyls and radicles divided by the number of measured seedlings, in accordance with the Association of Official Seed Analytsts - AOSA (2009). This way were obtained mean length of radicle, mean length of hypocotyl, and total length of the seedling, following recommendations made by both the ISTA as AOSA.

Dry mass of seedling: after obtaining seedling length, the hypocotyl-radicles axis were separated from the cotyledons, placed into Kraft paper bags previously identified, and placed into an oven, at 80 °C, for 24 h (Nakagawa, 1999), and immediately after the weighing was performed. Dry mass was obtained in milligrams and then divided by the number of seeds placed to germinate in each replicate (20), following the methodology recommended by ISTA (Hampton and TeKrony, 1995). Dry mass of each seedling was also assessed according to methodology described by AOSA (2009), i.e., by dividing the value obtained in the weighing by the number of seedlings assessed in each replication.

Hypocotyl/radicle ratio: for separately verifying the effect of the treatments on length of aerial part, as well as on length of the radicle of the seedlings, it was also computed the quotient between the measurements performed for these two components; once from this value it is possible to verify whether the development of one or other part of the seedling is more affected or less affected by size of the seed and/or by water stress. The expectation was that if there was any variation in the values from a treatment to another, and if such variation was proportionally distributed over the whole seedling, there would not be change in the hypocotyl/radicle ratio.

All experiments were performed using the completely randomized experimental design, and despite seeds of 10 different soybean genotypes had been used, compare them to each other was not the aim of this study. Thus, results obtained in all assessments were subjected to ANOVA, as if treatments had been arranged on a factorial 3 x 3 [3 seed sizes (P12, P13 and P14) x 3 water potentials (control, -0, 1 MPa, and -0.2 MPa)] with 40 repetitions; once data obtained for the 10 different genotypes were used in the statistical analyses as replications of the treatments. Thereby, the 10 genotypes were treated as a representative sample of the soybean cultivars most usually used to cultivation, and instead of four replications, the tests had 40 replications per treatment.

Treatment means of were compared by Tukey test, at 5% probability. Data analysis was performed using the statistical program GENES (Cruz, 2007); and before being analyzed percentage values were transformed to arcsine $\sqrt{x/100}$.

Results and Discussion

In the first count of germination to the control treatment (distilled water) there has been superiority in germination of the larger seeds over germination of the smaller seeds, corroborating results already found by Padua et al. (2010). However, such behavior was not repeated when the test was performed under water restriction conditions, since seeds sorted within the three sizes assessed (P12, P13, and P14) were affected similarly (Table 1). Nevertheless, in the final count of germination it was observed that the germination was reduced when the water potential was decreased; since as it may be seen on Table 1 there was a lower germination of the smaller seeds on the highest level of stress (-0.20 MPa). Data set on size of seeds, presented in that table shows that advantage of the larger seeds on the smaller ones has been punctual, what agrees with results obtained by Padua et al. (2010); who reported that under unfavorable conditions the highest amount of nutritional reserves available in the bigger seeds may signify greater resistance to adverse conditions during emergence seedlings in field.

As it was already expected, regardless the seed size, the intensification of water restriction has negatively affected as much their vigor as their germination; and as it was already verified by Moraes and Menezes (2003), as well as by Costa et al. (2004), under water restriction seed vigor was more highly affected than germination (Table 1). Within this context, Costa et al. (2012) had already demonstrated that the germination test, when performed at 25 °C, and under an osmotic potential of -0.1 MPa simulated with PEG 6000 may be used as a tool to assess vigor of soybean seeds; as it presents a result similar to that obtained through the accelerated aging test.

Vanzolini and Carvalho (2002) found that more vigorous seeds produce seedlings with higher length roots; a result that is similar to what was observed in this study. However, herein it was found that under ideal conditions the smaller seeds, identified in the first count of germination test as having lower vigor, produced seedlings with radicles of shorter length (Table 2). Nevertheless, when the test was performed under water potential of -0.20 MPa, the seedlings originating from smaller seeds have overcome the seedlings originated from bigger seeds in the length of hypocotyls and radicle, and hence in overall length.

Table 1. First and final count of germination (%) of soybean seeds classified by screens with three different mesh sizes (S12, S13, and S14) and germinated under different levels of osmotic potential (values presented in table represent mean of 40 replicates per treatment).

Osmotic Potencial (MPa)	First count of germination (%)			Final count of germination (%)		
0,0	80.85 Ba*	87.25 ABa	90.00 Aa	84.05 Aa	89.40 Aa	91.65 Aa
-0,1	44.60 Ab	42.70 Ab	41.95 Ab	73.70 Ab	74.20 Ab	71.00 Ab
-0,2	0.00 Ac	0.00 Ac	0.00 Ac	52.90 Bc	60.60 Ac	61.90 Ac
Screen[1]	S12	S13	S14	S12	S13	S14

*Means followed by the same uppercase letter in line and lowercase letter in column do not statistically differ between each other by the Tukey test, at 5% probability; [1]Metallic screens with oblong mesh in the sizes: 12 (12/64 x ¾"); 13 (13/64 x ¾"); and 14 (14/64 x ¾").

Table 2. Mean lengths of hypocotyl, radicle and seedling, obtained through two different methodologies (ISTA and AOSA), from seeds classified by screens with three different mesh sizes (S12, S13, and S14) and germinated under different levels of osmotic potential (values presented in table represent mean of 40 replications per treatment).

Osmotic Potencial (MPa)	ISTA methodology			AOSA methodology		
	Length of hypocotyl (cm.seedling $^{-1}$)					
0,0	8.987 Aa*	9.741 Aa	9.744 Aa	12.358 Aa	12.505 Aa	12.281 Aa
-0,1	2.687 Ab	2.559 Ab	2.466 Ab	3.690 Ab	3.538 Ab	3.469 Ab
-0,2	1.156 Ac	1.023 Ac	0.777 Ac	2.606 Ac	2.006 Bc	1.631 Bc
	Length of radicle (cm .seedling $^{-1}$)					
0,0	15.354 Ba	17.450 Aa	17.643 Aa	21.111 Aa	22.406 Aa	22.334 Aa
-0,1	11.395 Ab	11.154 Ab	10.548 Ab	15.772 Ab	15.294 Ab	14.660 Ab
-0,2	7.478 Ac	8.044 Ac	6.247 Ac	16.131 Ab	14.462 Bb	12.952 Cc
	Total length of seedling (cm. seedling^{-1})					
0,0	24.342 Ba	27.191 Aa	27.386 Aa	33.469 Aa	34.911 Aa	34.615 Aa
-0,1	14.082 Ab	13.713 Ab	13.014 Ab	19.462 Ab	18.832 Ab	18.129 Ab
-0,2	8.634 Ac	9.067 Ac	7.024 Ac	18.737 Ab	16.468 Bc	14.583 Cc
Screen[1]	S12	S13	S14	S12	S13	S14

*Means followed by the same uppercase letter in line and lowercase letter in column do not statistically differ between each other by the Tukey test, at 5% probability; [1]Metallic screens with oblong mesh in the sizes: 12 (12/64 x ¾"); 13 (13/64 x ¾"); and 14 (14/64 x ¾").

Beckert et al. (2000) have reported that the process of soaking of smaller seeds is faster and more intense, and that this may be explained by its smaller size what provides greater total surface contact with the moist soil, and that under conditions of water restriction the smaller seeds may be less affected; perhaps because even a small amount of water is already sufficient to activate germination process. Likewise, results obtained in this study show that when this test was performed under normal conditions, larger seeds have had a better and faster germination; however, when the test was carried out under water restriction, the smaller seeds were superior to larger seeds in relation to germinative process.

The dry matter accumulation in hypocotyl and radicle and, consequently, in the whole seedling, observed for seedlings originated from the larger seeds in control treatment, has exceeded dry mass accumulation observed in seedlings originating from the smaller seeds (Table 3); what had had already been observed by Padua et al. (2010). This fact can be explained by the greater availability of nutritional reserves existing in the bigger seeds, since the bigger this reserve the greater will be the seedling development, and consequently its dry mass; what was evidenced by the impairment undergone by the larger seeds when placed to germinate under water deficiency. Although on a lesser amount, the seedlings originated from smaller seeds have also had dry matter accumulation; however, regardless the seed size, this dry mass accumulation was statistically similar among all the different treatments; hence, leading to the confirmation of how severe were the impairments undergone by the seedlings originated from the larger seeds in relation to dry mass accumulation, when these seeds were placed to germinate under water deficit.

Table 3. Means of dry mass of the hypocotyl, radicle and seedling, obtained through two different methodologies (ISTA and AOSA), from seeds classified by screens with three different mesh sizes (S12, S13, and S14) and germinated under different levels of osmotic potential (values presented in table represent mean of 40 replications per treatment).

Osmotic potencial (MPa)	ISTA methodology			AOSA methodology		
	Dry mass of hypocotyl (mg .seedling $^{-1}$)					
0,0	19.643 Ca*	26.078 Ba	30.868 Aa	27.318 Ca	33.889 Ba	39.084 Aa
-0,1	9.540 Ab	9.199 Ab	8.848 Ab	13.128 Ab	12.673 Ab	12.494 Ab
-0,2	4.160 Ac	4.410 Ac	3.953 Ac	9.588 Ac	8.640 Ac	8.294 Ac
	Dry mass of radicle (mg .seedling $^{-1}$)					
0,0	8.436 Ca	11.103 Ba	13.080 Aa	11.539 Cab	14.184 Ba	16.430 Aa
-0,1	8.980 Aa	9.913 Aa	9.116 Ab	12.424 Aa	13.445 Aa	12.623 Ab
-0,2	4.883 Ab	6.214 Ab	5.396 Ac	10.740 Bb	12.007 Ab	11.22 ABc
	Dry mass of the whole seedling (mg .seedling $^{-1}$)					
0,0	28.079 Ca	37.181 Ba	43.947 Aa	38.856 Ca	48.073 Ba	55.513 Aa
-0,1	18.520 Ab	19.111 Ab	17.964 Ab	25.552 Ab	26.117 Ab	25.117 Ab
-0,2	9.042 Ac	10.624 Ac	9.349 Ac	20.329 Ac	20.646 Ac	19.517 Ac
Screen[1]	S12	S13	S14	S12	S13	S14

*Means followed by the same uppercase letter in line and lowercase letter in column do not statistically differ between each other by the Tukey test, at 5% probability; [1]Metallic screens with oblong mesh in the sizes: 12 (12/64 x ¾”); 13 (13/64 x ¾”); and 14 (14/64 x ¾”).

By analyzing the effects of water stress on seedling growth, both in length as in dry mass accumulation, it was observed that the higher the water restriction imposed to larger seeds, more severe were the impairments caused to seedling development. According to Moraes and Menezes (2003) if the hypocotyl and the radicle were proportionally affected there will be no statistical change in the ratio hypocotyl/radicle within the different treatments. Nevertheless, if there is a reduction of this value this means that the numerator of this fraction (the hypocotyl) has had a more sharply reduction; and if there is an increase this figure, in relation to experimental control, this means that the denominator (the radicle) was the most impaired.

Regardless seed size and considering both the length as the dry matter accumulation by seedlings, the hypocotyl was more affected by water restriction than the radicle (Table 4); and this can be explained by the fact that the ratio between hypocotyl/radicle had been lower to the extent that the water potential was lowered. Concerning the effect of seed size, the seedlings originating from the smaller seeds have shown higher values for the ratio hypocotyl/radicle as much in the control treatment as in the treatment with water potential of -0.2 MPa. This fact may have two alternative explanations, which are: or the seedlings originating from the smaller seeds have had best development of hypocotyl; or the seedlings originated from the larger seeds have had the radicle with longer length. Based on data presented on Table 2, it may be verified that under the water potential of -0.2 MPa, the seedlings originating from smaller seeds have shown that the three components assessed (hypocotyl, radicle, and the whole seedling) have had higher length than those lengths found for seedlings coming from larger seeds; thereby showing that the two alternatives are possible. Moreover, it is possible to verify in that table that the larger seeds had better performance in the control treatment, whereas smaller seeds have excelled only in treatments where there was water restriction.

Regarding dry mass accumulation by soybean seedlings it was possible to verify that there was no effect of seed size on the total length of seedlings; however, results achieved confirm the impairment caused to the hypocotyl, once the ratio between hypocotyl/radicle was reduced. On considering results obtained for the hypocotyl/radicle ratio, it is feasible to presume that under water deficit, the possibility exists that the seedling is able to choose between a larger development of radicle at expense of development of hypocotyl. Such event may be a natural strategy used by seedling to seek an alternative source of water to compensate for the deprivation caused by the water stress before fully developing the hypocotyl.

Table 4. Means of ratio between hypocotyl/radicle computed for length and dry mass of soybean seedlings originated from seeds classified by sieves with three different mesh sizes (S12, S13, and S14) and germinated under different levels of osmotic potential (values presented in table represent mean of 40 replications per treatment).

Osmotic Potencial (MPa)	Length			Dry mass		
0,0	0.590 Aa*	0.559 ABa	0.553 Ba	2.398 Aa	2.446 Aa	2.434 Aa
-0,1	0.239 Ab	0.236 Ab	0.242 Ab	1.083 Ab	0.956 Ab	1.028 Ab
-0,2	0.170 Ac	0.148 ABc	0.129 Bc	0.894 Ac	0.740 Ac	0.741 Ac
Screen[1]	S12	S13	S14	S12	S13	S14

*Means followed by the same uppercase letter in line and lowercase letter in column do not statistically differ between each other by the Tukey test, at 5% probability; [1]Metallic screens with oblong mesh in the sizes: 12 (12/64 x ¾”); 13 (13/64 x ¾”); and 14 (14/64 x ¾”).

Conclusions

Under ideal moisture conditions, soybean seeds with larger size present a higher germination speed and originate more vigorous seedlings.

Under osmotic potential of -0.2 MPa, soybean seeds with smaller size originate seedlings with hypocotyl and radicle with longer lengths.

Physiological quality of the soybean seeds is impaired by water stresses of -0.1 and -0.2 MPa, and in terms of seedling length and dry mass accumulation, the hypocotyl is more affected than the radicle.

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