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Research Article
Physiological Quality of Soybean Seed Cultivars (*Glycine max* (L.) Merr) with Different Maturity Groups

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Abstract

Background and Objective: Soybean is an important global commodity. As new soybean cultivars are released on an annual basis, cultivar-specific parameters related to maturity group can quickly became outdated. Therefore, the present study was designed to investigate the physiological qualities of sixteen soybean cultivars of three different maturity groups. **Materials and Methods:** Two experiments (2011-2012 and 2012-2013) were conducted with 16 cultivars of soybeans of different maturity groups in other to evaluate the hypothesis that maturity groups have a role in soybean physiological qualities. Seed germination, accelerated seed aging, cold tolerance, electrical conductivity and tetrazolium tests were performed. Data were summarized and subjected to analysis of variance (one-way ANOVA) and Tukey HSD test where differences were found as a mean comparison test. Correlation matrices and multivariate analysis (PCA, HCA and PLS-R) were also applied for dimensionality reduction seeking the most important variables related to seed physiological qualities. **Results:** Significant statistical differences ($p<0.05$) were found in all cultivars in germination capacity, aging, cold tolerance, electrical conductivity, seed vigor, seed viability, moisture and stink bug damage. Super early cultivars (SYN 3358, SYN 1163, BMX ENERGIA, EXP 9039) presented good physiological qualities (vigor, viability, cold tolerance and germination capacity). FUNDACEP 66, NIDERA 4766 and EXP 810 RR presented lower qualities. **Conclusion:** Seed vigor, aging, cold tolerance and viability were the main variables affecting physiological quality independent of maturity group. Higher vigor, seed viability and germination capacity was found in super early cultivars.

Key words: Maturity groups, non-supervised techniques, physiological quality, predictive models, seed germination, soybeans

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) seeds have high levels of protein (38-40%), oil (18-20%) and are broadly utilized by the food industry as either raw or processed forms of various soy products[1]. Based on the type of soy product desired, soybeans are harvested at various maturity stages, from pod to dried seeds. A wide range of contents and quality of metabolites may exist due to the maturity differences of the harvested soybeans[1]. The crop is a major contributor to the world's food supply[2]. Besides the importance, there are many factors that influence seed physiological potential of soybeans such as germination (viability) and vigor which govern the theoretical capacity of seeds to express their vital functions under both favorable and unfavorable environmental conditions[3].

Growth, development and yield of soybeans depend on genetic potential of a cultivar and its interaction with the environment. In a field situation, nature provides the major portion of the environmental influence on soybean development and yield, however, soybean producers can manipulate this environment with proven managerial practices[4-6]. The criteria for selecting soybean varieties depend on yield potential, stand ability, pest resistance and maturity. With the exception of yield, which reflects the total response of a variety to a set of growing conditions, maturity is the most important criterion in selecting a variety. If a variety is too early or too late at a location, it will be limited in potential performance[5]. During maturation, soybean seeds undergo many changes, including color, weight, size and metabolic profiles[1]. The classification for maturity is based upon the adaptability of a soybean variety to effectively utilize the growing season in a given region. An early maturing variety may develop fewer leaves or progress through the different stages at a faster rate, especially when planted late. A late maturing variety may develop more leaves or progress more slowly. The rate of plant development for any variety is directly related to temperature, so the length of time between the different stages will vary as the temperature varies both between and within the growing season. Deficiencies of nutrients, moisture, or other stress conditions may lengthen the time between vegetative stages but shorten the time between reproductive stages[5]. These changes and variability may have significant impacts on phenology, growth and yield of soybeans. Effective strategies to adapt agricultural production require deep understanding of soybean maturity groups. Unfortunately, today there is no universally accepted single test for assessing seed physiological potential of a given species or group of species to achieve predetermined objectives. For this reason, research on seed testing and the identification of factors that affect seed performance have been considered priorities among seed scientists in comparison to other quality attributes.

Rapid and uniform emergence of vigorous seedlings of the desired cultivar is key events to ensure high plant performance that affects uniformity of development, yield and quality of the harvested product. These factors emphasize the importance of selecting high quality seed lots that are available in sufficient quantities to meet demand[3].

When a producer changes relative maturity group (MG) in soybean, irrigation requirements and the amount of nitrogen fixation that takes place during production year, among a host of other impacts, are affected[7]. Combining maturity types, sowing dates, growers manipulate the timing of critical periods and accommodate crops in cropping sequences[8]. Variations in maturity group and sowing date modify plant size and architecture. Late maturity groups have an extended crop cycle in relation to early ones, mainly during the vegetative stages. This increases the number of nodes per plant, shoot mass and leaf area index. By contrast, delaying sowing with respect to the optimum reduces the duration of the vegetative period, which reduces shoot mass, node number and modifies plant architecture. These differences in crop phenology and morphology caused by variations in maturity group or sowing date also influence the accumulation of shoot biomass from crop emergence to flowering[8]. Planting early, semi-early and super-early soybean maturity group cultivars under several years and its effect on physiological qualities have not been fully investigated, mainly those developed by Brazilian agricultural research company (EMBRAPA) and Santa Catarina agricultural research and rural extension company (EPAGRI).

The main goals of this research were to assess physiological qualities (seed germination, seed viability, accelerated seed aging capacity, cold tolerance capacity, electrical conductivity, seed vigor, seed viability, mechanical damage, moisture damage and damage by stink bugs) on sixteen cultivars classified as of three maturity groups (early, semi and super early cycle) under two harvest periods (2011-2012 and 2012-2013).

MATERIALS AND METHODS

Geographical characteristics of the study area: The experiment was conducted under field conditions in a commercial farming, in Campos Novos County, Santa Catarina, Southern Brazil, during 2 harvests 2011-2012 and 2012-2013. According to climatological atlas of Santa Catarina, Campos

Table 1: Characteristics of the soybean cultivars used in the experiment, during 2011-2012 and 2012-2013 harvests

Commercial name	Official name (RNC)	Maturity group	Portuguese classification	English classification	Weight of 1000 seeds (g)
ND 4725 RG	A 4725RG	4.7	Super Precoce	Super early	172.4
BMX ENERGIA RR	BMX ENERGIA RR	5.0	Super Precoce	Super early	176.0
BMX VELOZ RR	5953 RSF	5.0	Super Precoce	Super early	172.0
BMX APOLO RR	Don Mario 6.8 i	5.5	Super Precoce	Super early	168.0
FUND 62 RR	FUNDACEP 62RR	5.6	Super Precoce	Super early	146.0
BMX TURBO RR	BMX TURBO RR	5.8	Super Precoce	Super early	205.2
SYN 3358 RR	SYN3358 RR	5.8	Super Precoce	Super early	180.0
SYN 1059 RR	SYN1059 RR	5.9	Precoce	Early	177.7
BMX FORÇA RR	BMX Força RR	6.2	Precoce	Early	174.0
BMX MAGNA RR	Don Mario 7.0i	6.2	Precoce	Early	143.0
FUND 66 RR	FUNDACEP 66RR	6.2	Precoce	Early	200.0
BMX TORNADO RR	6863 RSF	6.2	Precoce	Early	175.0
SYN 1163 RR	SYN1163 RR	6.3	Semi Precoce	Semi early	185.3
A 6411 RG	A 6411RG	6.4	Semi Precoce	Semi early	191.5
FUND 60 RR	FUNDACEP 60RR	6.4	Semi Precoce	Semi early	139.0
BMX POTÊNCIA RR	BMX Potência RR	6.7	Semi Precoce	Semi early	168.0

Novos is located in the middle West of Santa Catarina, with average altitude of 964.23 m, 27°24' South latitude and 51°12' West longitude, annual mean temperature of 16.5°C and mean annual rainfall of 1851.7 mm[9].

On-farm trials: The 1st experiment (2011-2012) used seeds of category 1 (C1-higher seed vigor) previously assigned to Ø 6.5 mm sieve and the 2nd experiment (2012-2013) used seeds from previous crop harvesting, certified to category 2 (C2-high vigor). Seeds were harvested in November of each year for 1st and 2nd experiment. Each experimental plot was composed of 5 lines spaced at 0.5 m with 13 m length. The area of each plot (8 m^2) was formed by the three central rows, excluding 0.5 m from each end. 15 seeds were used per linear meter, in a total a density of 30 plants m^2. The fertilization was carried out according to the chemical analysis of the soil, in accordance with the technical recommendations of the crop in the region. Sixteen cultivars were used with different maturity groups (GM), all with indeterminate growth habit. Of these, seven cultivars classified as super early (GM 4.7-5.8), five as early-cycle (GM 5.9 to 6.2) and four as semi early (GM 6.3-6.7). Table 1 summarizes the cultivars used in the experiments represented by their commercial and official name according to national register of plant varieties (RNC) and the weight of 1000 seeds.

Laboratory analysis

Sampling: Seeds of the useful area of each plot in each repetition in the field (named as simple samples), were collected (700 g) and joined to obtain composite sample approximately to 2000 g. From this, 1000 g (sample) were sampled and divided into 4 sub samples, which were labeled as working samples (e.g., samples required to carry out all laboratory tests) according to Brasil[10].

Seed germination test (ge): The germination test was conducted using four replications of 50 seeds, wrapped in paper roller of type "*Germitest*" dampened with distilled water at a ratio of 2.5 times the mass of the dry paper, under constant temperature of 25°C, at germination chamber. The analyses of seed germination were performed 6 days after the beginning of the test, as described by Brasil[10].

Accelerated seed aging test: The accelerated aging test was conducted according to Filho *et al.*[11], with 4 replications of 50 seeds each. The seeds were distributed in single and uniform layer on stainless steel screen set in the interior of the plastic box type "seed dispersal" containing 40 mL of distilled water. The boxes were capped and kept for 48 h in aging chamber with 100% relative humidity and temperature of 41°C[10].

Cold tolerance capacity of the seeds: For cold tolerance, 4 repetitions of 50 seeds were used, using the same test procedure of germination test (item 2.3.2). Rolls with the seeds were sealed in plastic bags and placed in a climate chamber at a temperature of 10°C for 5 days. After this period, the rolls were removed from the plastic bags and transferred to the germination at 25°C. The number of normal seedlings was evaluated in a single score on the 4th day after the transfer to the ambient temperature[12].

Electrical conductivity: The electrical conductivity test was performed with 4 replications of 50 seeds packed in container containing 75 mL of ultrapure water and maintained at 25°C in a germinator during 24 h. After it, the electrical conductivity of the solution was determined and the results were expressed as $\mu S\ cm^{-1}\ g^{-1}$.

Seed vigor, seed viability, mechanical, moisture and stink bug damages (tetrazolium test): For seed vigor, seed viability, mechanical damage, moisture and stink bug damages (also called tetrazolium test), 100 seeds were used (2 repetitions of 50 seeds/treatment), which were placed in a Germitest paper moistened 2.5 times the mass of the paper, during 16 h at 25°C in germinator. Then, the seeds were placed in plastic container and kept submerged in a solution of 0.075% of 2, 3, 5 triphenyl tetrazolium, at 40°C, in the dark, for 3 h. After this time, seeds were washed and kept submerged in water until the time of the evaluation[13].

Data analysis and statistics: Data were summarized and tested if they follow a Gaussian model (normal distribution) using Shapiro-Wilk test (5% of probability) and homogeneity of variances between groups and within groups, using the Levene test (5% of probability). Data were transformed using arc sine for all variables. Parametric analysis of variance (one-way ANOVA) was used[14]. Tukey HSD test was used where differences were found as a mean comparison test. Correlation matrices were also performed. Non-supervised multivariate analysis (PCA, HCA and seriated cluster heatmaps) were also applied to the data for dimensionality reduction seeking the most important variables related to seed physiological qualities and cultivar similarities. Supervised predictive model (decision trees) and PLS-R were also applied aiming to understand the importance of variables related to seed germination. All analysis were performed in R software (R Core team)[14], using scripts produced by the research group and an R report (for data reproducibility) in html format, datasets are made available as supporting data.

RESULTS

Seed germination: Results of the study are summarized in the Table 2 and 3 (a-b) for 1st and 2nd experiment, respectively. According to one-way analysis of variance (ANOVA, $p<0.05$) differences were found in all variables studied for our 1st experiment (2011-2012, Table 2a-b). Using the Tukey HSD test, significant statistical differences in germination ($p<0.05$) capacity were found in the cultivars studied. High seed germination was observed for the cultivar SYN3358 (Table 2a). FUNDACEP 66 presented the lower germination. The first cultivar is from a super early maturity group and the last belonging to early group of maturity. In the 2nd experiment different trend was observed, BMX APOLO, FUNDACEP 62, SYN1059 and BMX FORÇA RR presented high germination and NIDERA 4725RR and FUNDACEP 66 lower germination capacity (Table 3a). Except for SYN1059, all others cultivars that exhibited higher germination belongs to super early group of maturity.

Seed aging: The aging test provides valuable information on storage and seedling field emergence potentials. Seeds are hydrated to a specific level when exposed to relatively high temperature (40-45°C, usually 41°C) and humidity (around 100% Relative Humidity-RH) Following this aging treatment, seeds are subjected to a germination test and higher vigor seed lots tolerate this aging condition better than lower vigor seed lots and produce a higher percentage of normal seedlings. Results of our seed aging capacity were higher for SYN 3358RR and lower for FUNDACEP 66 (Table 2a). Results of our 2nd experiment showed the cultivars BMX APOLO, FUNDACEP 62 and SYN 1059 to exhibit high aging capacity and FUNDACEP 66 the lower value (Table 3a). Results showed many super early cultivars to have high seed aging capacity and germination at lower variance explained by our regression model.

Cold tolerance: Regarding the cold tolerance variable, SYN 3358RR presented the major capacity of cold tolerance than others and again in FUNDACEP 66 cultivar was observed the lower capacity of cold tolerance (Table 2a). In the 2nd season of harvest (2nd experiment) high cold tolerance was observed for BXM ENERGIA and lower values for FUNDACEP 66 and NIDERA 4725 (Table 3a).

Electrical conductivity: Electrical conductivity were superior in EXP 810RR, FUNDACEP 66 and NIDERA 4725RR cultivars and lower in SYN 3358RR, SYN 1059RR cultivars (Table 2a). For 2nd experiment, NIDERA 4725 presented high value of electrical conductivity and BMX POTENCIA and FUNDACEP 60 the lower values of that variable (Table 3a).

Vigor, viability, moisture damage, mechanical and stink bug damage: Tetrazolium is a rapid test to estimate seed viability and vigor based on color alterations of seed living tissues in contact with a solution of 2, 3, 5 triphenyl tetrazolium chloride, thus reflecting the degree of activity of the dehydrogenase enzyme system closely related to seed respiration and viability. Results of tetrazolium test for the 1st season of harvest (experiment) showed higher vigor for SYN 3358RR and lower for EXP 810RR and FUNDACEP 66 (Table 2b). Seed viability was superior for BMX MAGNA RR, BMX POTÊNCIA RR, EXP 9039, SYN 3358 and lower for NIDERA 4725 RR and EXP 810 RR (Table 2b). Seed mechanical

Table 2: Means followed by standard error of the mean of the 1st experiment (2011-2012 harvest), (a) For germination, aging capacity, cold tolerance and conductivity, (b) For viability, mechanical damage, moisture damage and stink bug damage

Cultivar	Cycle	Ge	Aging	Cold	Cond
BMX_FORÇA_RR	Early	95.50 ± 1.89^{ab}	90.00 ± 1.41^{ade}	79.50 ± 3.20^{acd}	52.72 ± 1.07^{cf}
BMX_MAGNA_RR	Early	91.00 ± 4.43^{ab}	92.50 ± 1.71^{ad}	77.00 ± 2.08^{ace}	51.91 ± 1.23^{cf}
EXP_9039	Early	91.00 ± 1.29^{ac}	90.00 ± 2.94^{ad}	87.50 ± 1.50^{ab}	50.43 ± 1.81^{def}
FUNDACEP_66	Early	78.00 ± 4.08^{c}	72.00 ± 4.24^{f}	$56.50\pm2.22g$	73.84 ± 3.86^{ab}
SYN_1059_RR	Early	95.00 ± 1.29^{ab}	95.50 ± 0.96^{ab}	81.00 ± 1.73^{acd}	41.04 ± 0.46^{f}
A6411_RG	Semi_early	93.00 ± 1.00^{ac}	$85.50\pm3.40b^{cdf}$	61.50 ± 5.32^{fg}	57.17 ± 1.42^{ce}
BMX_POTÊNCIA_RR	Semi_early	94.00 ± 0.82^{ac}	93.50 ± 1.26^{ac}	85.00 ± 2.65^{acd}	45.54 ± 0.45^{def}
FUNDACEP_60	Semi_early	93.50 ± 1.50^{ab}	78.50 ± 1.71^{cdf}	62.50 ± 2.36^{eg}	60.75 ± 3.14^{bcd}
SYN_1163_RR	Semi_early	96.00 ± 1.63^{ab}	90.00 ± 2.71^{ad}	72.00 ± 0.82^{def}	47.93 ± 0.91^{def}
BMX_APOLO	Super_early	94.00 ± 0.82^{ab}	87.50 ± 6.55^{adf}	77.50 ± 0.50^{ace}	57.24 ± 1.47^{ce}
BMX_ENERGIA_RR	Super_early	90.00 ± 1.41^{ac}	93.00 ± 0.58^{ad}	64.00 ± 0.82^{eg}	66.04 ± 2.60^{ac}
BMX_TURBO_RR	Super_early	96.00 ± 0.82^{ab}	90.50 ± 1.50^{ad}	63.50 ± 1.71^{eg}	50.66 ± 2.55^{cf}
EXP_810_RR	Super_early	90.00 ± 1.15^{bc}	77.50 ± 3.30^{df}	75.50 ± 3.77^{bcef}	76.60 ± 0.79^{a}
FUNDACEP_62_RR	Super_early	92.00 ± 2.45^{ac}	91.50 ± 2.63^{ad}	86.00 ± 2.16^{ac}	51.68 ± 1.63^{cf}
NIDERA_4725RR	Super_early	88.00 ± 1.83^{bc}	72.50 ± 5.44^{ef}	73.50 ± 4.19^{cef}	73.32 ± 7.77^{ab}
SYN_3358_RR	Super_early	98.00 ± 1.15^{a}	97.50 ± 1.26^{a}	88.00 ± 2.94^{a}	43.45 ± 1.45^{ef}
p-value**		0.19	0.23	0.55	0.00
CV (%)		6.56	7.28	6.15	7.25

Cultivar	Cycle	Vigor	Via	MeD	MoD	SBD
BMX_FORÇA_RR	Early	88.00 ± 0.00^{bd}	97.00 ± 0.58^{abc}	12.00 ± 0.00^{ce}	43.00 ± 0.58^{i}	10.00 ± 1.15^{de}
BMX_MAGNA_RR	Early	90.00 ± 0.00^{bd}	99.00 ± 0.58^{a}	14.00 ± 2.31^{ce}	66.00 ± 0.00^{ef}	2.00 ± 1.15^{f}
EXP_9039	Early	93.00 ± 1.73^{ab}	99.00 ± 0.58^{a}	10.00 ± 2.31^{de}	67.00 ± 5.20^{def}	6.00 ± 1.15^{ef}
FUNDACEP_66	Early	60.00 ± 2.31^{f}	95.00 ± 0.58^{ad}	31.00 ± 1.73^{a}	97.00 ± 0.58^{a}	5.00 ± 1.73^{ef}
SYN_1059_RR	Early	87.00 ± 1.73^{bd}	98.00 ± 1.15^{ab}	17.00 ± 0.58^{bce}	62.00 ± 2.31^{fg}	5.00 ± 1.73^{ef}
A6411_RG	Semi_early	84.00 ± 1.15^{cde}	98.00 ± 0.00^{ab}	21.00 ± 2.89^{acd}	75.00 ± 1.73^{ce}	11.00 ± 0.58^{de}
BMX_POTÊNCIA_RR	Semi_early	92.00 ± 1.15^{bc}	99.00 ± 0.58^{a}	9.00 ± 0.58^{e}	49.00 ± 1.73^{hi}	10.00 ± 1.15^{de}
FUNDACEP_60	Semi_early	82.00 ± 1.15^{de}	90.00 ± 1.15^{cde}	13.00 ± 0.58^{ce}	76.00 ± 1.15^{cd}	9.00 ± 0.58^{de}
SYN_1163_RR	Semi_early	88.00 ± 1.15^{bd}	98.00 ± 0.00^{ab}	10.00 ± 1.15^{de}	54.00 ± 2.31^{gh}	5.00 ± 0.58^{ef}
BMX_APOLO	Super_early	82.00 ± 0.00^{de}	94.00 ± 0.00^{bd}	14.00 ± 2.31^{ce}	73.00 ± 0.58^{ce}	19.00 ± 5.20^{bcd}
BMX_ENERGIA_RR	Super_early	85.00 ± 0.58^{bd}	94.00 ± 0.00^{bd}	12.00 ± 3.46^{ce}	67.00 ± 0.58^{def}	34.00 ± 3.46^{a}
BMX_TURBO_RR	Super_early	85.00 ± 0.58^{bd}	98.00 ± 1.15^{ab}	23.00 ± 2.89^{ac}	59.00 ± 0.58^{fh}	18.00 ± 1.15^{bcd}
EXP_810_RR	Super_early	60.00 ± 1.15^{f}	83.00 ± 1.73^{e}	31.00 ± 1.73^{a}	88.00 ± 1.15^{b}	25.00 ± 0.58^{ac}
FUNDACEP_62_RR	Super_early	88.00 ± 1.15^{bd}	98.00 ± 1.15^{ab}	23.00 ± 5.20^{ac}	75.00 ± 0.58^{ce}	12.00 ± 1.15^{de}
NIDERA_4725RR	Super_early	73.00 ± 2.89^{ef}	85.00 ± 1.73^{de}	28.00 ± 3.46^{ab}	94.00 ± 1.15^{a}	30.00 ± 3.46^{ab}
SYN_3358_RR	Super_early	96.00 ± 2.31^{a}	99.00 ± 0.58^{a}	15.00 ± 0.58^{bce}	79.00 ± 0.58^{c}	13.00 ± 1.73^{ce}
p-value**		0.41	0.00	0.14	0.02	0.39
CV(%)		24.24	4.94	14.97	4.00	18.78

**p-value of Shapiro-Wilk test of normality. Different letters after means in the column represent significant differences between cultivars ($p<0.05$) according to Tukey HSD test. Ge: Germination capacity, Aging: Aging capacity, Cold: Cold tolerance and Cond: Electrical conductivity. The acronyms Via:Viability, MeD: Mechanical damage, MoD: Moisture damage and SBD: Stink bug damage

damage was higher in EXP 810 RR and FUNDACEP 66 and lower for BMX POTÊNCIA RR. Moisture damage was observed to be higher in FUNDACEP 66, NIDERA 4725 RR and lower for BMX FORÇA RR. Stink bug damage was higher in BMX ENERGIA RR and lower in BMX MAGNA RR (Table 2b).

Results of the 2nd experiment (2012-2013) with same cultivars are summarized in the Table 3b. One-way ANOVA showed significant differences ($p<0.05$) in all variables except for stink bug damage. As it can be observed in Table 3b regarding the vigor, FUNDACEP 60RR and SYN 1163 presented high values. Three different groups were found. Lower vigor was observed for NIDERA 4725 and all other remain similar. Seeds of cultivars FUNDACEP 66, NIDERA 4725 and FUNDACEP 62 were highly influenced by mechanical damage (Table 3b). Moisture damage was also high in NIDERA 4725 and FUNDACEP 66 cultivars and lower for BMX POTENCIA RR.

DISCUSSION

High quality of seeds is reflected directly on the yield of the crop, providing uniformity of the population, high vigor of seedlings and plants, absence of pathogens transmitted by seeds and consequently, greater production. Therefore, evaluations which allow obtaining reliable information about the physiological potential of seed are of fundamental importance for decision making to be taken during the production[15]. The results clearly demonstrate super early cultivars to have good physiological quality as observed for

Table 3: Means followed by standard error of the mean of the 2nd experiment (2012-2013 harvest), (a) For germination, aging capacity, cold tolerance and conductivity, (b) For viability, mechanical damage, moisture damage and stink bug damage

Cultivar	Cycle	Germination	Aging	Cold	Conductivity
A6411_RG	Semi_early	94.00±1.41^{ab}	85.00±5.07^{ac}	92.00±0.00^{ac}	55.19±1.71^{de}
BMX_APOLO	Super_early	99.00±0.58^{a}	97.00±1.29^{a}	96.00±1.63^{ac}	73.84±2.75^{bd}
BMX_ENERGIA_RR	Super_early	97.00±0.58^{ab}	94.50±0.96^{ab}	95.50±2.06^{a}	64.19±1.20^{be}
BMX_FORÇA_RR	Early	97.00±1.29^{a}	93.50±0.96^{ab}	94.00±1.15^{ac}	62.00±0.25^{cde}
BMX_MAGNA_RR	Early	95.50±0.96^{ab}	72.00±3.56^{bc}	89.00±1.29^{ac}	55.97±2.56^{cde}
BMX_POTÊNCIA_RR	Super_early	94.00±1.63^{ab}	89.50±1.26^{ab}	91.50±1.89^{ac}	51.46±1.43^{e}
BMX_TURBO_RR	Super_early	96.50±0.50^{ab}	94.50±0.96^{ab}	83.00±4.04^{ac}	59.96±5.98^{be}
EXP_810_RR	Super_early	92.00±0.00^{ab}	80.00±0.82^{ac}	86.00±1.41^{bcd}	83.37±4.38^{b}
EXP_9039	Super_early	92.50±0.50^{ab}	88.50±4.50^{ac}	94.50±1.26^{ac}	58.91±0.94^{cde}
FUNDACEP_60	Semi_early	97.00±1.73^{ab}	90.50±1.71^{ac}	93.00±1.73^{ac}	48.63±1.37^{e}
FUNDACEP_62_RR	Super_early	99.00±0.58^{a}	99.00±0.58^{a}	93.50±0.96^{ac}	73.65±1.98^{bc}
FUNDACEP_66	Early	77.00±2.08^{b}	56.00±2.94^{c}	78.00±2.45^{cd}	66.19±1.69^{be}
NIDERA_4725RR	Super_early	82.50±3.40^{b}	78.50±2.50^{ac}	66.00±3.83^{d}	105.54±7.22^{a}
SYN_1059_RR	Early	98.50±0.96^{a}	96.50±1.26^{a}	90.50±2.50^{ac}	51.49±1.25^{cde}
SYN_1163_RR	Semi_early	95.50±0.96^{ab}	96.50±1.50^{ab}	96.50±0.96^{ab}	55.70±2.02^{cde}
SYN_3358_RR	Super_early	95.00±0.58^{ab}	95.50±1.71^{ab}	92.00±0.82^{ac}	54.87±2.58^{cde}
p-value**		0.95	0.83	0.80	0.04
CV (%)		5.73	8.90	5.13	5.07

Cultivar	Cycle	Vigor	Viability	MeD	MoD	SBD
A6411_RG	Semi_early	94.00±2.00^{ab}	100.00±0.00^{a}	0.00±0.00^{b}	32.00±2.00^{cf}	14.00±0.00^{a}
BMX_APOLO	Super_early	97.00±1.00^{ab}	100.00±0.00^{a}	0.00±0.00^{b}	19.00±3.00^{fg}	13.00±1.00^{a}
BMX_ENERGIA_RR	Super_early	95.00±5.00^{ab}	100.00±0.00^{a}	2.00±2.00^{b}	37.00±3.00^{bcde}	8.00±2.00^{a}
BMX_FORÇA_RR	Early	96.00±2.00^{ab}	99.00±1.00^{a}	0.00±0.00^{b}	17.00±1.00^{fg}	14.00±4.00^{a}
BMX_MAGNA_RR	Early	96.00±2.00^{ab}	99.00±1.00^{a}	1.00±1.00^{b}	31.00±1.00^{cf}	4.00±2.00^{a}
BMX_POTÊNCIA_RR	Semi_early	95.00±1.00^{ab}	99.00±1.00^{a}	0.00±0.00^{b}	10.00±2.00^{g}	17.00±1.00^{a}
BMX_TURBO_RR	Super_early	96.00±0.00^{ab}	99.00±1.00^{a}	2.00±0.00^{b}	28.00±6.00^{df}	6.00±0.00^{a}
EXP_810_RR	Super_early	92.00±4.00^{ab}	97.00±1.00^{a}	0.00±0.00^{b}	47.00±3.00^{acd}	19.00±9.00^{a}
EXP_9039	Early	97.00±1.00^{ab}	99.00±1.00^{a}	0.00±0.00^{b}	22.00±2.00^{efg}	4.00±2.00^{a}
FUNDACEP_60	Semi_early	98.00±2.00^{a}	100.00±0.00^{a}	0.00±0.00^{b}	16.00±2.00^{fg}	12.00±2.00^{a}
FUNDACEP_62_RR	Super_early	96.00±2.00^{ab}	99.00±1.00^{a}	4.00±2.00^{ab}	37.00±3.00^{bcde}	8.00±2.00^{a}
FUNDACEP_66	Early	86.50±5.50^{ab}	99.00±1.00^{a}	17.00±1.00^{a}	56.00±4.00^{ab}	8.00±8.00^{a}
NIDERA_4725RR	Super_early	77.00±3.00^{b}	97.00±1.00^{a}	6.00±4.00^{ab}	66.00±2.00^{a}	13.00±1.00^{a}
SYN_1059_RR	Early	95.00±3.00^{ab}	99.00±1.00^{a}	0.00±0.00^{b}	40.00±4.00^{bcde}	11.00±1.00^{a}
SYN_1163_RR	Semi_early	98.00±2.00^{a}	100.00±0.00^{a}	0.00±0.00^{b}	30.00±6.00^{cf}	2.00±2.00^{a}
SYN_3358_RR	Super_early	95.00±1.00^{ab}	100.00±0.00^{a}	2.00±2.00^{b}	49.00±1.00^{ac}	9.00±1.00^{a}
p-value**		0.97	0.00	0.00	0.00	0.07
CV(%)		7.33	4.79	85.23	30.78	32.89

**p-value of Shapiro-Wilk test of normality. Different letters after means in the column represent significant differences between cultivars ($p<0.05$) according to Tukey HSD test. The acronyms MeD: Mechanical damage, MoD: Moisture damage and SBD: Stink bug damage

seed germination, vigor, viability and cold tolerance. Mengistu and Heatherly[16] found different results in soybean cultivars of different maturity groups. High germination of seed was found in late cultivars. Gwathmey *et al*.[17], reported that seed maturity is a complex polygenic trait, influenced by genetic and environmental factors that affect morphological, phenological and physiological attributes of the plant. Those reported factors may act in different manner between cultivars and geographical area. According to Kandpal *et al*.[18], seed viability and vigor are the two most important parameters directly related to seed germination performance and seedling emergence.

During storage, seeds undergo physiological and physico-chemical changes, termed aging, including deterioration of the chemical component of the seeds. However, with prolonged storage, seed viability decreases with the change in lipid peroxidation. The rate at which the seed ages depends on its ability to resist degradation and on its protective mechanisms. Our results showed some super early cultivars with good storage and field seeding potentials. Previous studies[18] have defined seed viability as the development of the seed embryo and the ability to produce normal seedlings under favorable conditions (i.e., moisture, humidity, mechanical resistance, temperature), while seed vigor comprises those attributes that determine the potential for rapid, uniform development of normal seedlings under different field conditions. Therefore a good quality seed has strong vigor and steady germination and establishes quickly in the field. In general, seeds start to lose vigor before they lose their ability to germinate, therefore, vigor (germination potential) testing is an important practice in seed production programs. Some of the conventional methods used to estimate seed viability and vigor include the germination test, the tetrazolium test and the electric conductivity test[18]. As reported by Filho[3], the evaluation of germination and the identification of seed lots of high performance is an important initiative towards successful crop production. As reported by Filho[3], maximum physiological potential is achieved close to seed maturity and just after this stage, seeds become prone to deterioration depending on harvest time, environmental conditions and procedures adopted for seed drying, processing and storage. The most obvious manifestation of initial seed aging is the decline in germination speed of viable seeds followed by a decrease in seedling size and an increased incidence of abnormal seedlings (although the primary root protrusion rate should remains high). The low germination rate has been associated to early signs of membrane disorganization while the occurrence of seedling abnormalities (in the final stages of deterioration) is attributed to the significant death of tissues in different seed parts, particularly in meristematic tissues. Panabianco *et al.*[19]., reported an inverse relationship between seed aging and germination. Differences may be duo to genetic factors of cultivars and environmental conditions where the experiment was done. Stanisavljevic *et al.*[20]., also recorded a significant reduction ($p<0.05$, Tukey test) in seed germination during seed aging. Another similar result was found by Li *et al.*[21], who reported that seed aging can delay germination of seeds.

The cold test is considered the oldest vigor test method and probably the most popular used for seed vigor assessment in the United States. The procedure has the objective to evaluate the response of seed samples subjected to a combination of low temperature, high substrate water content and if possible, presence of pathogens. Two types of stress prevail in this test: (i) Suboptimal temperature favors leakage of cell solutes during seed water uptake due to the disorganized configuration of the membrane systems. In such situation, membrane repair is relatively slow, increasing the release of leachates, including sugars and (ii) Presence of microorganisms when the substrate includes soil is increased not only as a consequence of the exposure to low temperature but also stimulated by the release of sugars so that damage to seed performance is enhanced. These conditions contribute to a reduction in the speed and percentage germination or seedling emergence, depending on the procedure adopted to conduct the test. Consequently, the vigor of a seed lot is proportional to the degree of seed survival when exposed to such an unfavorable environment[3]. Physiological mechanisms involved with cold tolerance at the vegetative period are better understood. Among the many processes, can be cited[22] (the regulating role of kinases dependent on calcium and activated by cold temperature exposition, the involvement of abscisic acid as a signaling molecule and the levels of activation of the enzymes associated to oxidative stress such as ascorbate peroxidase, catalase and glutathione reductase)[22]. Interestingly in this study, super early cultivars showed high cold tolerance. Due to the negative effects of low temperature on growth, cold tolerance is an important feature for both, temperate and high altitude, regions[23,24]. Cultivars with high capacity of cold tolerance exhibited high germination rate than others.

The electrical conductivity (EC) test is classified as a biochemical test. Low germinability of timothy seeds has been associated to the high release of solutes during hydration. Later, electrical conductivity of cotton seed soaking solution was found to be inversely proportional to germination but until now there was no mention of this analysis with possible evaluation of seed vigor. The principle of the EC test is that less vigorous or more deteriorated seeds show a lower speed of cell membrane repair during seed water uptake for germination and therefore release greater amounts of solutes to the external environment. The loss of leachate includes sugars, amino acids, fatty acids, proteins, enzymes and inorganic ions (K^+, Ca^{+2}, Mg^{+2}, Na^+ and Mn^{+2}) and the test evaluates the amount of ion leakage. Under field conditions, leakage of exudates after sowing, reflecting the loss of cell membrane organization and selective permeability, can stimulate the growth of pathogenic microorganisms and impair seedling emergence[3]. Our results are consistent of those reported in the literature. Cultivars with high EC showed lower germination capacity. Except for NIDERA 4725 cultivar, all other that showed high EC are of early group of maturity. Ramos *et al.*[25], also showed an inverse relationship between EC and seed viability. In their research, seeds with high EC were non-viable. In our research FUNDACEP 66 presented lower germination and seed viability. As reported by Fessel *et al.*[26], EC test determines indirectly the integrity of seed membrane systems and is used for assessment of seed vigor, because the test detects the seed deterioration process since its early phase. Those authors also reported that storage period and temperature can influence the conductivity.

Long *et al.*[27], reported that a key determinant of seed persistence is inherent seed longevity, which is a complex expression of physiological traits including cellular mobility, internal protective compounds and the ability of cells to resist and repair damage. The two main factors that influence biochemical aging reactions in seeds are seed water activity and temperature. Seed water activity is influenced by the

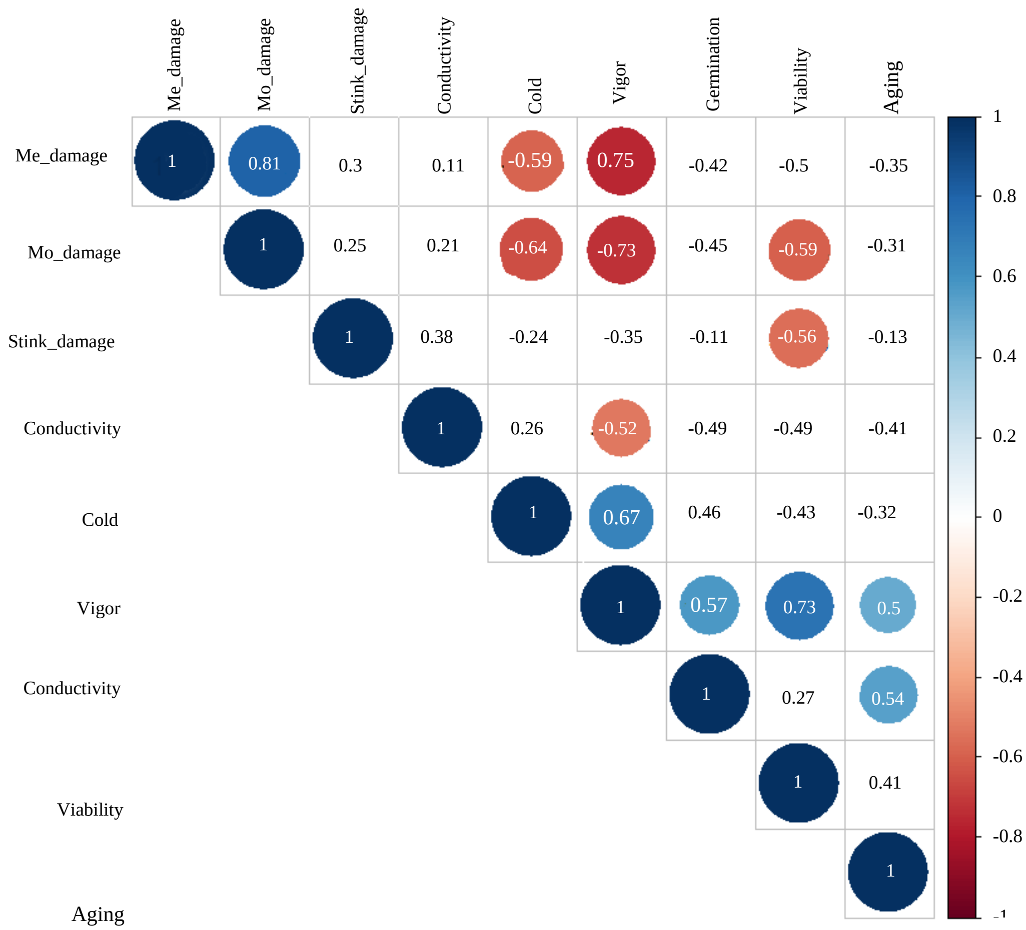

Fig. 1: Association (Pearson correlation, 5%) between all variables studied. Me_damage = mechanical damage and Mo_damage = moisture damage

humidity (or water potential), temperature of a seed's environment and by seed lipid content. Seed water activity and temperature influence the viscosity of the cytosol, membrane fluidity and integrity, the activity of antioxidants, rates of transcription and translation. All those mentioned factors may have influenced the EC of our tested cultivars.

Interestingly, the cultivars with high mechanical damage, stink bug damage in our research presented lower viability and germination and high EC, confirming our previous hypothesis of an inverse relationship between these variables with seed viability. Previous research[28] suggested that loss of seed viability may result from pathogens or physiological factors and from biochemical changes which are accelerated by high temperature and high relative humidity. Super early cultivars showed good physiological qualities and these results can be used to cultivar selection. As reported by Liu *et al.*[29], the step of cultivar selection is important for the successful soybean management. A meta-analysis conducted by Rotundo and Westgate[30] in soybean seeds indicated that genetically factors of seeds may be affected by environmental factors and a greater knowledge of the physiological processes that regulate physiological qualities of seeds is essential. Seed physiological qualities were reported to decrease by increase in temperature[31]. Cera and co-workers[32] studying cultivar maturity and potential yield of soybeans concluded that early and later cultivars may have equal potential if exposed to similar environment. Similar responses in seed filling were also found during shade stress in early and later cultivars[33]. All these results indicate that despite the group of maturity, many other factors must be included when the selection of cultivars is aimed.

Correlation matrix and regression models: When data were correlated (Pearson correlation) to see if there are an association between the variables, interesting results were observed (Fig. 1). Germination was positively correlated with seed aging, cold tolerance, vigor and seed viability (r = 0.54, 0.46, 0.57, 0.27, respectively) and negatively correlated with conductivity, mechanical damage, moisture damage and stink bug damage (r = -0.49, -0.42, -0.45 and -0.11, respectively). A positive correlation of vigor with viability, cold tolerance and mechanical damage was found. High negative correlation was also found between vigor with mechanical and moisture damage.

Multivariate analysis

Non-supervised techniques: Principal component analysis: As previously reported by Sills and Gossett[34] and Uarrota *et al.*[35], chemometric techniques that include multivariate models (e.g., principal component analysis, hierarchical cluster analysis, partial least squares discriminant analysis (PLS-DA), linear discriminant analysis (LDA), decision trees (DTs) and support vector machines (SVM)) can be applied to complex and collinear data to extract relevant information. Both non-supervised (PCA) and supervised (PLS-DA, LDA, DTs and SVM) methods reduce large datasets by combining collinear variables into a small number of latent variables (LVs), which are then used in place of the full dataset to build predictive models.

A third approach applied to the data was non-supervised multivariate analysis (PCA, HCA) for dimensionality reduction of the data and verify similarities between cultivars of different maturity groups. When PCA was applied to the data (Fig. 2a), the total variance explained by 1st and 2nd component of the model was 87.24%. The 1st component (PC1) explained 70.58% and the 2nd (PC2) 16.67%. Most cultivars were grouped in the 1st component duo to their similarities in vigor and viability. Four distinct groups of cultivars according to their similarities can be observed. NIDERA and EXP 810 grouped together in the (PC1+/PC2+) duo to their high values of electrical conductivity and high values of stink bug damage, BMX ENERGIA, BMX APOLO, FUNDACEP 60, BMX FORÇA formed a 3rd group in the PC1-/PC2+ duo to their values of germination, aging capacity and cold tolerance. FUNDACEP 66 separated alone in the PC1+/PC2- duo to the high values of moisture and stink bug damage. The loading values and scores of the PCA showed that the most variables that influenced the cultivars in the PC1+ were mechanical damage (36%) followed by conductivity and moisture damage (35%). PC1- was most influenced by vigor (39%), followed by aging (34%) and germination (33%). In the PC2+, samples were grouped together duo to their high values of stink bug damage (67%) followed by germination, conductivity and aging (34, 33 and 32%, respectively). Finally, the variables that most contributed in the PC2- were the viability (39%) and mechanical damage (17%).

Aiming to understand similarities between maturity groups, a second PCA was done (Fig. 2b). Interestingly, early cycle and semi early cultivars grouped together in the PC1 and PC2- and super early cultivars were different from others and grouped in the PC2-/PC2+. The variables that positively contributed to the clustering of early and semi early cultivars were vigor, viability, cold tolerance, conductivity and stink bug damage and negatively were the variables mechanical and moisture damage.

Hierarchical cluster dendrogram: When data were subjected to second approach of data clustering using a hierarchical dendrogram analysis (HCA), Similar results from those found in PCA analysis were observed but at this time with good visualization of similarities. The cophenetic correlation was 96%. As it can be seen in the HCA (Fig. 3), cultivar EXP 810 grouped alone but was most correlated to NIDERA and FUNDACEP 66. Similar results were observed for BMX MAGNA. Three main groups were observed: (SYN 3358, SYN 1163, SYN1059, BMX FORÇA, BMX POTÊNCIA and EXP 9039-group 1), (BMX TURBO, A6411, FUNDACEP 60-group 2) and (BMX ENERGIA, BMX APOLO and FUNDACEP 62-group 3). Similarities in the HCA were mostly duo to vigor, viability and germination. The variables that most influenced FUNDACEP 66 were viability, mechanical damage and vigor. NIDERA and EXP 810 grouped together duo to conductivity, germination, seed viability, aging and cold tolerance.

Supervised techniques: Decision tree and PLS-R: As previously reported in the literature[34,35], chemometric techniques that include multivariate models (e.g., principal component analysis (PCA), hierarchical cluster analysis (HCA), partial least squares discriminant analysis (PLS-DA), linear discriminant analysis (LDA), partial least square regression (PLS-R) and support vector machines (SVM)) can be applied to complex and collinear data to extract relevant information. Both non supervised (HCA, PCA) and supervised (PLS-DA, PLSR, LDA and SVM) methods reduce large datasets by combining collinear variables into a small number of latent variables (LVs), which are then used in place of the full dataset to build prediction models[34,35].

Decision tree model and partial least square regression was used taking into account the germination capacity as the target variable and verify the most related variables to germination of the cultivars. Decision tree model (Fig. 4)

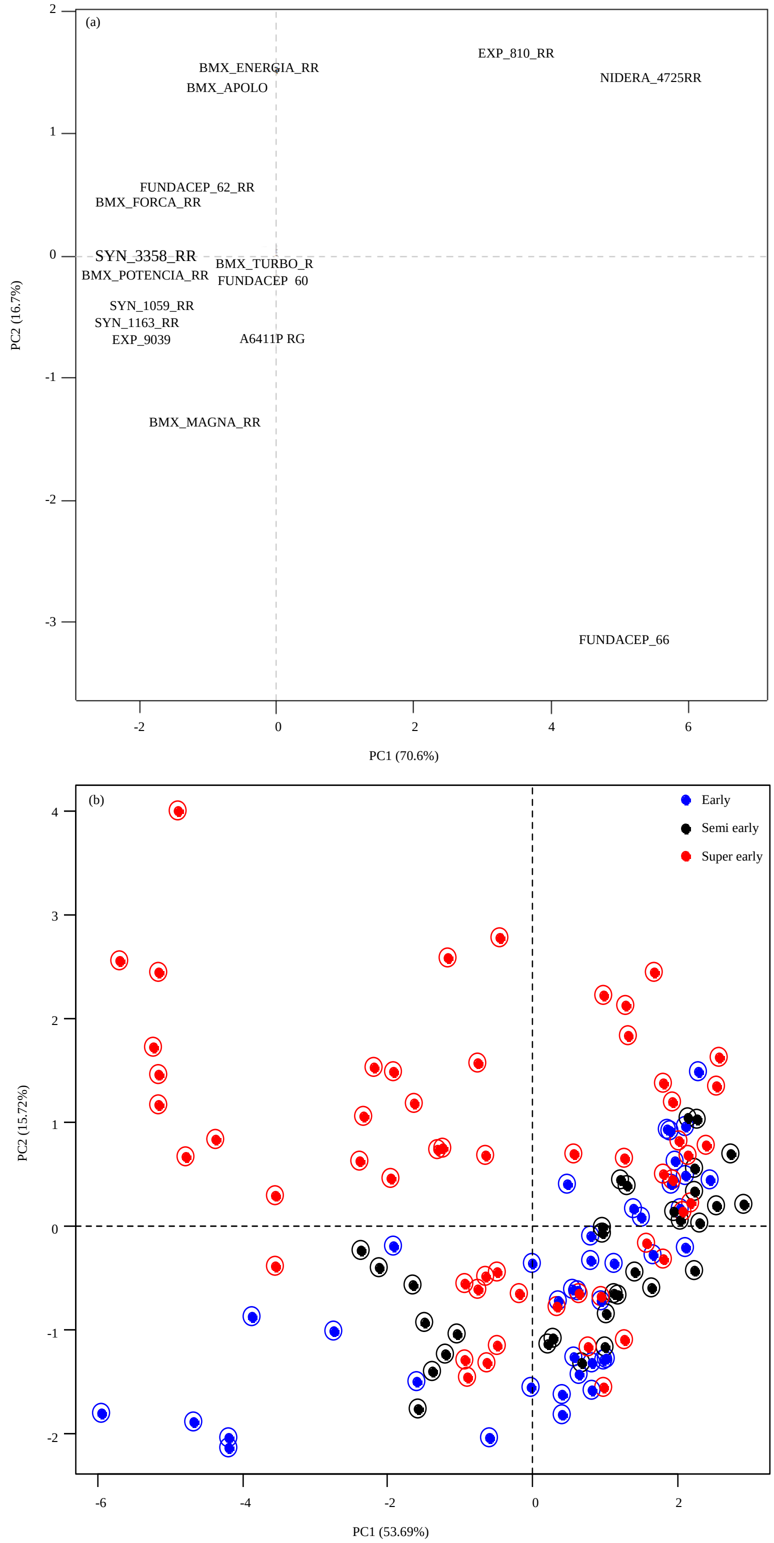

Fig. 2(a-b): (a) Scores plot of principal component analysis using cultivars as factor and (b) PCA using maturity groups as factor

showed that the vigor is highly correlated with germination, followed by seed aging, mechanical damage and conductivity.

When a partial least square regression was used to the dataset, taking germination as target and seeking correlation

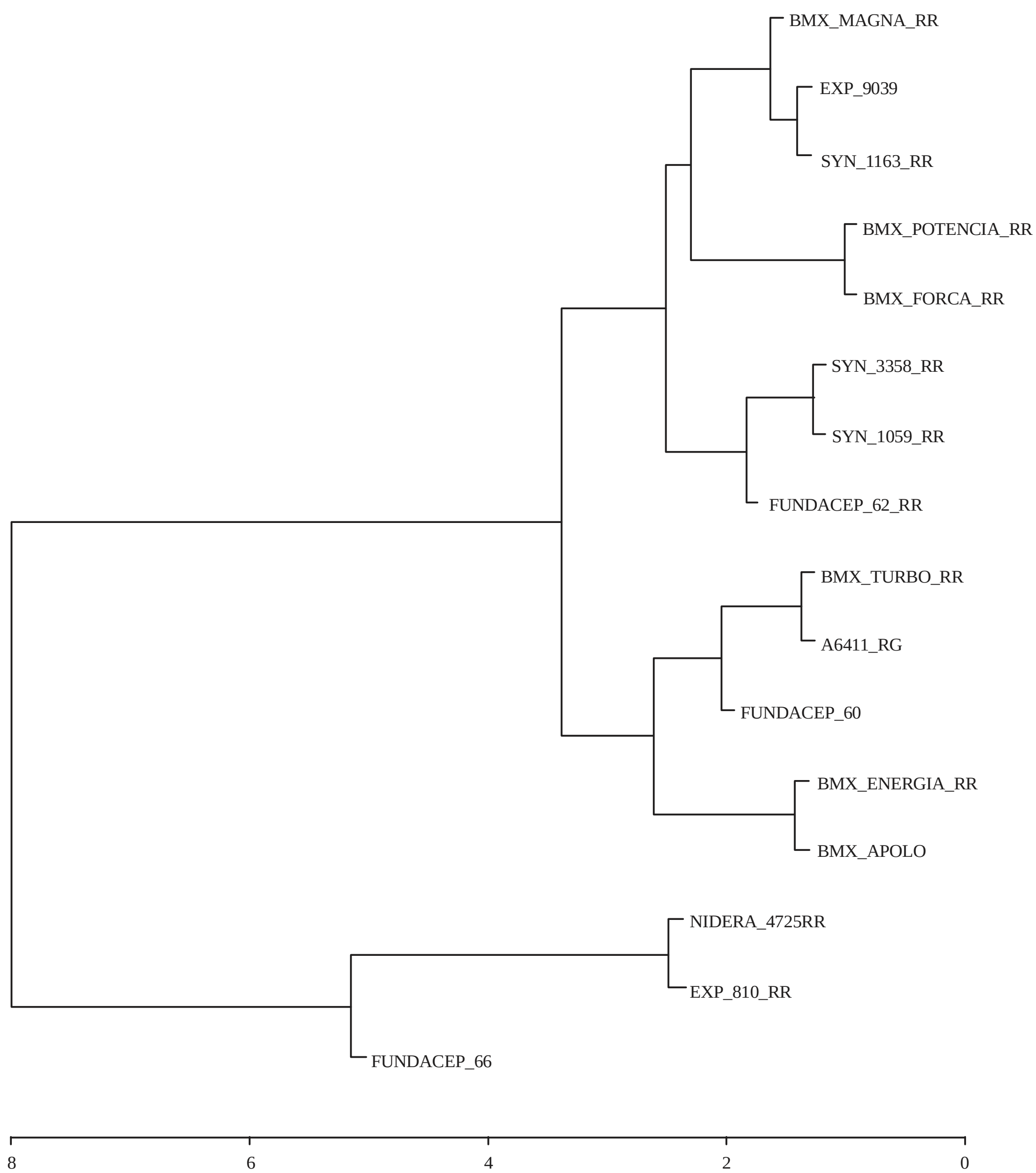

Fig. 3: Hierarchical cluster dendrogram of the variables studied showing similarities between them

Table 4: Score loadings of partial least square regression showing the correlation of predictors with the response variable (Germination) for the first 3 components and the explained variance captured by each variable

	PC1*	PC2	PC3	Variance
Aging	0.3283441	0.52507513	-0.540184384	0.4912520
Cold tolerance	0.3749515	-0.01864400	0.327164906	0.5390170
Conductivity	-0.2974630	-0.31546180	-0.071172305	0.3072082
Vigor	0.4708086	-0.04844206	-0.005430929	0.8416848
Viability	0.3892226	-0.43144938	-0.705508927	0.5623900
Mechanical damage	-0.3897832	0.25824257	-0.361548592	0.5669350
Moisture damage	-0.4034811	0.24821496	-0.275102876	0.6048199
Stink bug damage	-0.2187823	0.62715026	-0.268563145	0.1696395

*PC: Principal component. PC1, PC2 and PC3 indicate the correlation of the predictors with germination capacity of the seeds evaluated

between predictors and the target, an interesting finding was observed (Table 4). The PLSR using a cross-validation of leave-one-out (LOO) model showed that the most correlated variables to seed germination in the first component or latent

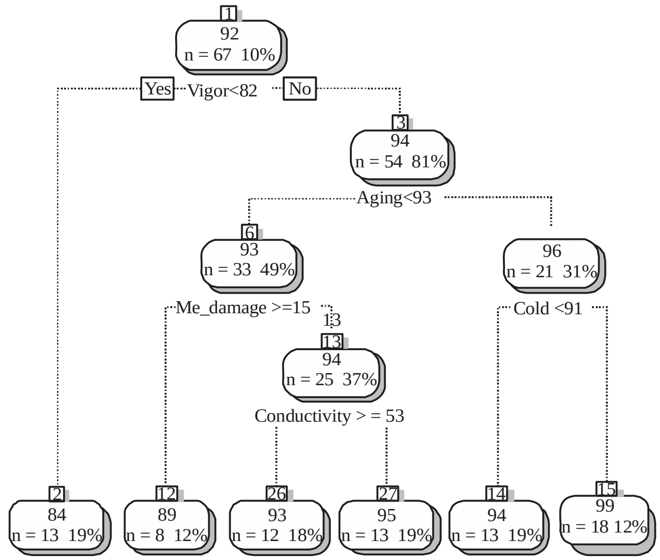

Fig. 4: Decision tree model showing the hierarchy of variables related to seed germination. All variables were used as predictors except germination which was used as target variable

variable are: Vigor (47%), viability (39%), cold tolerance (37%) and aging capacity (33%). Seed germination was negatively correlated to moisture damage (40%) followed by mechanical damage, electrical conductivity and stink bug damage (38, 30 and 22%, respectively). The total variance explained by the model was 75.36%, being 56.89 for latent variable 1 (axis 1 or PC1) and 27.59% for second latent variable. The predicted values by the model were similar to those found by our research.

CONCLUSIONS

Super early cultivars presented high vigor, viability and germination, lower conductivity, mechanical, moisture and stink bug damages. All cultivars presented high germination except FUNDACEP 66 (75.5%) and good viability (>88%). The SYN 3358 has high cold tolerance and cultivars (BMX POTÊNCIA, EXP 9039, SYN 1163 and SYN 3358) have high vigor. Cultivars (EXP 810, NIDERA and FUNDACEP 66) suffer from mechanical, moisture and stink bug damage. Decision tree model related germination with vigor, aging and conductivity. The PLSR confirmed that vigor, viability, aging and cold tolerance are positively correlated with seed germination and mechanical damage, moisture, conductivity and stink bug damage can highly reduce seed germination.

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SIGNIFICANT STATEMENT

This study discovered that super early cultivars have high physiological qualities. Multivariate techniques showed that seed vigor, viability, aging and cold tolerance are the main variables related to seed germination. Thus, physiological attributes and multivariate techniques can be used as a tool for agronomists, breeders and farmers for selecting high quality seed lots and cultivars with high performance.

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