1 **Seed reserve mobilization and seedling morphology in a bioassay for the**

2 **detection of genetically modified soybean**

3

4 Abstract - Germination and initial seedling development are physiological processes that
5 determine the establishment of crops. During heterotrophic growth, each seedling develops
6 from the biomass of its seeds. Thus, verifying the potential of genotypes to mobilize reserves
7 under stress has been important. The aim of this study was to investigate how glyphosate
8 affects the mobilization of reserves and seedling morphology. Two tolerant and two
9 herbicide-sensitive cultivars were submitted to germination, seedling length and reserve
10 mobilization tests, including treatments with glyphosate solutions (0, 0.06 and 0.12 %). The
11 hypocotyl / radicle ratio and the efficiency of conversion of reserves to seedlings were also
12 verified. The data were submitted to analysis of variance and test of means. It was observed
13 that the percentage of normal seedlings and length seedlings were affected due to the
14 concentration of the herbicide in the treatments, being the consequences more pronounced for
15 sensitive cultivars; the glyphosate-tolerant genotype and with the best physiological quality
16 mobilized more reserves and was more efficient in converting biomass to seedlings; in
17 morphology, the average length of the seedlings was reduced due to the herbicide, being the
18 roots affected in such a way that they became smaller than the hypocotyls. The herbicide
19 affects the morphology of the seedlings mainly the radicle, and the mobilization of the
20 reserves discriminates the genotypes regarding tolerance to glyphosate.
21 Keywords: dynamics of seed reserve, *Glycine max*, herbicide.
22

23   **Introduction**

24   The germination test (BRASIL, 2009) is one of the first analyses carried out on a seed

25 lot and is performed to assess the germinative potential of the seeds. However, the

26 germination rate observed in the test does not guarantee the establishment of a crop,

27 especially in adverse environmental situations, when the mobilization of seed reserves for

28 seedlings is not satisfactory (OLIVEIRA et al., 2020). In this respect, high vigor seeds are

29 desired by producers because they emerge faster and more uniformly from the soil,

30 accumulate more dry mass in the plants, which are also higher (HENNING et al., 2010).

31   The initial stages of the seedling development coincide with the period of crop

32 establishment, moment of great apprehension of farmers, aware of the relationship between

33 the formation of the stand and high yield (HENNING et al., 2010). Well-consolidated stand

34 increases soil cover, reduces evaporation of available water, reflecting positively on

35 productivity, improving the competitive power of crops against weeds (DIAS et al., 2011).

36   According to Mohammadi et al. (2011), the initial weight of the seed, the mobilized

37 fraction of reserves, and the efficiency in converting these reserves to dry matter are relevant

38 components to be evaluated during the heterotrophic growth of the seedlings. During this

39 stage, it is very important to observe how and how much of the seed reserves are mobilized

40   under favorable or unfavorable scenarios for a crop. Studies showed that more vigorous seeds

41   have increased their capacity to mobilize reserves, and consequently, a better initial

42   performance (HENNING et al., 2010; OLIVEIRA et al., 2020).

43   Crops face adversities from planting to harvest, with germination and seedling

44   emergence being considered the most critical and potentially most sensitive stages to

45   environmental stresses (ALI et al., 2018). Many examples may be presented. In wheat, the

46   seedlings length, the weight of the mobilized reserve, and the fraction of use of this reserve

47   become reduced under water and salt stress (SOLTANI; GHOLIPOOR; ZEINALI, 2006). In

48   soybeans, under water stress, the seedling is impaired, being the development of the

49   hypocotyl is more affected than the radicle (PEREIRA; PEREIRA; DIAS, 2013). The use of

50   desiccants in crops can affect the germination and vigor of harvested soybean seeds, with a

51   negative influence on the mobilization of reserves (DELGADO; COELHO; BUBA, 2015).

52   Copper causes negative effects on cell elongation and cell division in *Vicia sativa* roots and

53   inhibits the mobilization of proteins from cotyledons (MUCCIFORA; BELLANI, 2013). In

54   general, the effects are proportional to the intensity of the stress, which increases the

55   consumption of reserves, without this meaning conversion into dry seedling mass

56   (OLIVEIRA et al., 2020). On the other hand, the pretreatment of soybean seeds with 'cold

57   plasma' improved the germination rate, length and dry weight of the seedlings, as well as the

58   mobilization of seed reserves and efficiency in the use of reserves (LING et al., 2014).

59   Projects on the mobilization of reserves contribute to the understanding of the

60   potential for acclimatization of seedlings (SILVA; AZEVEDO NETO; GHEYI, 2019).

61   Expanding knowledge about the process of mobilizing seed reserves, under the most diverse

62   contexts, can contribute to a better understanding of this important stage. Our research group

63   realized that the availability of genetically modified cultivars for glyphosate tolerance is a

64   good model for studies on the effects of herbicides on the initial development of seedlings.

65   Considered as a model species, our research indeed brings relevant information for soybean

66   and other crops species.

67   Given the above, the objective of this work was to study the dynamics of soybean seed

68   reserves during the heterotrophic development of the seedlings and their morphology, in an

69   artificial environment simulating the physiological stress that soybean seeds face during

70   germination.

71

72   **Materials and Methods**

73   **Water content**

74   To measure the water content (WC) of the studied seed lots, three replicates of 50

75   seeds (W1) were weighed, taken to the oven at 105 ° C for 24 hours, and weighed again (W2)

76   on a scale with four decimal places. Based on these data, the following formula was applied

77   (SOLTANI; GHOLIPOOR; ZEINALI, 2006).

$$WC = [(W2 - W1) / W1] * 100$$

Germination test

80   The germination test was carried out according to the recommendations of the

81   Regulation for Seed Analysis (BRASIL, 2009), with adaptations (PEREIRA et al., 2009a), to

82   obtain the first count and the final germination count. Four soybean cultivars were analyzed, 2

83   sensitive (BRS511, EMGOPA315) and 2 glyphosate-tolerant (TMG1264, P98Y11). The

84   samples were submitted to germination in substrate moistened with water in the control

85   treatment and solutions of the herbicide (0.06 and 0.12%) in the other treatments (PEREIRA

86   et al., 2009a), in the proportion of 2.5 times the weight of dry paper (BRASIL, 2009). A

87   completely randomized design was adopted, in a factorial scheme, composed of 4 genotypes x

88   3 doses of the herbicide, with 4 replications.

Seedling length test

90   The seedling length test was carried out according to (PEREIRA et al., 2009b), with

91   adaptations regarding the moistening of the substrate, with three solutions of the herbicide (0,

92   0.06 and 0.12% e. a.). A completely randomized design was adopted, in a factorial scheme, 3

93   cultivars x 3 doses of the herbicide, with 10 repetitions, with each experimental plot

94   consisting of 10 seeds. After the rolls confection, they were packed in plastic bags, with the

95   open end for ventilation, and kept in BOD for 7 days at 25 ° C. At the end of the test, the

96   average lengths of seedling (ALS), hypocotyl (ALH) and radicle (ALR) of each experimental

97   plot were obtained, using a millimeter ruler.

98   From the average lengths, the proportions of hypocotyl and root of the seedlings in

99   each treatment were known, using the following equations:

100   Hypocotyl Proportion (% H / S) = (ALH / ALS) x 100

101   Radicle Proportion (% R / S) = (ALR / ALS) x 100

102   Additionally, the percentages of reduction observed for hypocotyl (RDH) and radicle

103   (RDR) were obtained, due the treatments. For this, the average lengths of the hypocotyl and

104   radicle, under treatment with the herbicide, were compared with those of the experimental

105   control. The following equations were used:

106   Hypocotyl reduction [0 to 0.06%] = [1 - (ALH 0.06% / ALH 0%)] * 100

107   Radicle reduction [0 to 0.06%] = [1 - (ALR 0.06% / ALR 0%)] * 100

3

108   Hypocotyl reduction [0 to 0.12%] = [1 - (0.12% ALH / 0% ALH)] * 100

109   Radicle reduction [0 to 0.12%] = [1 - (ALR 0.12% / ALR 0%)] * 100

110 **Reserves Mobilization and Efficiency in Conversion of Reserve to Seedling**

111   Previously, the 120 experimental plots (10 seeds each) separated for the seedling

112 length test were weighed on a precision scale, providing the weight of 10 seeds (W.10) in

113 micrograms. As the water content of each seed lot was previously obtained, it supported the

114 estimation of the dry reserve weight (DW.10) in each experimental plot or in each seed

115 (W.1S) for mobilization from the seeds to seedlings during germination (PEREIRA;

116 PEREIRA; DIAS, 2015).

117   After measuring the seedlings lengths, the cotyledons, hypocotyls, and radicles of each

118 experimental plot were separated with a stylus and collected in their respective envelopes.

119 Then, these envelopes were kept in an oven at a regulated temperature of 80 °C for 24 hours.

120 From that period, the contents of the envelopes were weighed and the data, in micrograms

121 (μg), used to analyze the mobilization of reserves (PEREIRA; PEREIRA; DIAS, 2015).

122   Considering the number of seedlings evaluated, the average weight of the dry matter

123 of the hypocotyl (W.1H), radicle (W.1R), seedling (W.1Sd), and cotyledon pair (W.2C) of

124 each experimental plot was obtained. From this information, the first aspect analyzed was the

125 rate of reduction of the seed reserve (% SRR). Given the prior knowledge of the dry weight of

126 the seed (W.1S) and the dry weight of the pair of cotyledons (W.1PC), it was possible to

127 quantify the amount of seed mobilized reserve.

128 $$\%SRR = (W.1S - W.1PC) / W.1S.$$

129   From the knowledge of the dry weight of hypocotyl (W.1H), radicle (W.1R), and

130 seedling (W.1Sd), as well as the reserve that was mobilized from the seeds (SRR = W.1S –

131 W.1PC), it was possible to calculate the proportion of reserve mobilized to the hypocotyl

132 (RMH), radicle (RMR) and seedling (RMS).

133 $$RMH = (W.1H / SRR) \times 100$$

134 $$RMR = (W.1R / SRR) \times 100$$

135 $$RMS = (W.1Sd / SRR) \times 100$$

136   Additionally, we obtained information about the Efficiency of the Conversion of

137 Reserve into Seedling (ERCS). Unlike the variable mobilized reserve for seedling (%SRR),

138 which considers how much of the mobilized reserve has become a seedling, the ECRS

139 variable considers how much of the reserve contained in the seed has become a seedling.

140 $$ECRS = (W.1Sd / W.1S) \times 100$$

4

141   Finally, from the lengths and weights of the seedlings, it was possible to obtain the

142   linear weights of hypocotyl (LWH), radicle (LWR) and seedling (LWS) in μg / cm.

$$LWH = W.1H / ALH$$

$$LWR = W.1R / ALR$$

$$LWH = W.1Sd / ALS$$

Experimental design and data analysis

147   The tests were performed in a completely randomized design, in a factorial scheme.

148   The number of repetitions and the size of the experimental plots varied between experiments.

149   The data were subjected to analysis of variance and means compared by the Scott-Knott test,

150   at the level of 5% probability.

151

Results and Discussion

Germination test

154   Analyzing the first and the final counts of germination data (Table 1), it was notable

155   that both herbicide solutions (0.06, and 0.12 %) inhibited the development of normal

156   seedlings by the genotypes, like observed also by Bervald et al. (2010) and Pereira et al.,

157   (2018). In the case of glyphosate-tolerant cultivars, the highest concentration of the herbicide

158   (0.12 %) affected the first and the final counts of germination, as observed by Pereira et al.,

159   (2018), however, the glyphosate-tolerant seedlings developed secondary roots, while the

160   sensitive ones did not (Figure 1). The absence of secondary roots in glyphosate-sensitive

161   seedlings is a striking feature of this bioassay (BERVALD et al., 2010; MELO; FAGIOLI;

162   SÁ, 2013; PÁDUA et al., 2012). According to Albrecht et al. (2014), the physiological quality

163   and the vigor of the lots fall due to the concentration of the herbicide.

164

Table 1. First count and final count of the germination of four soybean cultivars submitted to germination test on moistened substrate with glyphosate solutions.

Dosage	First Count of Germination (%)				Final Count of Germination (%)			
	Emgopa 315	UFUS 7415	P98Y11	TMG 1264	Emgopa 315	UFUS 7415	P98Y11	TMG 1264
0.00 %	76.5 Ba	52.0 Ca	54.0 Ca	91.5 Aa	78.0 Ba	56.5 Ca	59.5 Ca	94.0 Aa
0.06 %	0.5 Cb	0.0 Cb	58.0 Ba	80.5 Ab	1.5 Cb	0.1 Cb	60.5 Ba	94.0 Aa
0.12 %	0.0 Bb	0.0 Bb	5.5 Bb	62.0 Ac	0.5 Cb	0.1 Cb	27.5 Bb	78.0 Ab
Mean	37.4				45.4			
CV(%)	26.7				20.4			

* Averages followed by the same letter, uppercase in the horizontal and lowercase in the vertical, do not differ each other, at the level of 5 % probability.
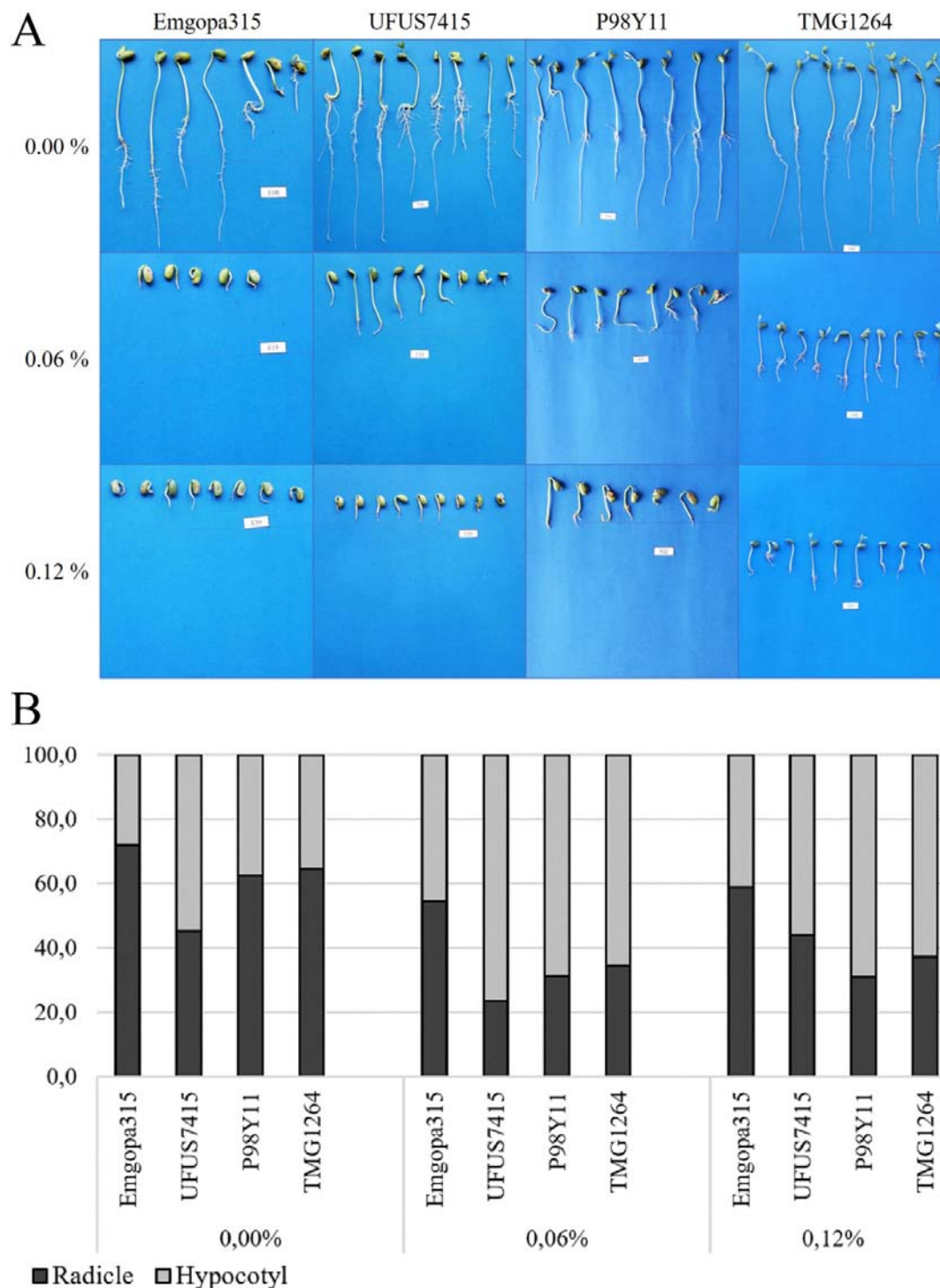
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Figure 1. Seedling morphology (A), hypocotyl and radicle proportions (B) of Emgopa315, UFUS7415, P98Y11, and TMG1264 cultivars, submitted to germination in substrate moistened with different glyphosate solutions (0, 0.06 and 0.12 %).

In a work assessing the efficiency of toothpeeks mostened with glyphosate to diferentiate tolerant and non-tolerant soybean cultivars, Silva et al. (2015) stated that the

173   injured plants of transgenic cultivars keeped growing the hypocotyl (main stem) and

174   developed new lateral branches instead of new lateral radicles like in our research. This

175   differenc may be explained because their role plants were not exposed to glyphosate at the

176   initial growing stages, then the glyphosate followed the xylem flux to the aerial parts of the

177   seedlings. Differently, our seedlings were completely exposed to the glyphosate solution

178   treatments, then we can affirm the radicule tissues are more sensitive to glyphosate, since no

179   secondary branches were observed in our results.

180   **Seedling length test**

181   The herbicide treatments reduced the lengths of the hypocotyl (ALH), radicle (ALR),

182   and seedling (ALS) (Table 2). In the herbicide treatments, the secondary roots are absent or

183   incipient in sensitive seedlings, with an aspect of blocked development. Cultivars that carry

184   the CP4 EPSPS gene are glyphosate-tolerant (NAKATANI et al., 2014). When these cultivars

185   are submitted to germination in substrate moistened with glyphosate solutions, secondary

186   roots are present, but in length, number, and volume smaller than in the control treatment, as

187   observed by Pereira et al., (2018); in fact, the herbicide affects the development of sensitive

188   and tolerant seedlings at different levels of intensity.

Table 2. Average length of hypocotyl, radicle and seedling of four soybean cultivars treated with three (0, 0.06, and 0.12 %) doses of glyphosate.

Average length of the hypocotyl - ALH (cm)			
	Emgopa 315	UFUS 7415	P98Y11	TMG 1264
0.00 %	5.12 Ca	7.89 Aa	6.79 Ba	7.54 Aa
0.06 %	1.66 Cb	3.65 Bb	5.09 Ab	5.67 Ab
0.12 %	1.22 Bb	1.42 Bc	3.83 Ac	3.87 Ac
Mean	4,5			
CV(%)	18,8			
Average length of the radicle - ALR (cm)			
	Emgopa 315	UFUS 7415	P98Y11	TMG 1264
0.00 %	13.2 Aa	6.48 Ca	11.3 Ba	13.5 Aa
0.06 %	1.91 Bb	1.15 Bb	2.28 Ab	3.03 Ab
0.12 %	1.74 Ab	1.08 Ab	1.68 Ab	2.33 Ab
Mean	4,9			
CV (%)	31,3			
Average length of the seedling - ALS (cm)			
	Emgopa 315	UFUS 7415	P98Y11	TMG 1264
0.00 %	18.3 Ba	14.4 Ca	18.1 Ba	21.0 Aa
0.06 %	3.56 Bb	4.80 Bb	7.38 Ab	8.71 Ab
0.12 %	2.96 Bb	2.50 Bc	5.51 Ac	6.19 Ac
Mean	9,4			
CV (%)	17,4			

* Averages followed by the same letter, uppercase in the horizontal and lowercase in the vertical, do not differ, at the level of 5 % probability.

189

190   According to Funguetto et al. (2004); Heinz; Viegas Neto and Valente (2011); Pereira

191   et al. 2018; and Tillmann and West (2004) the length reductions are more striking for

192   glyphosate-sensitive genotypes. In our study, this was a notable occurrence.

193   Once it was verified the average lengths (ALH, ALR, and ALP) are reduced, it was

194   questioned whether treatments with the herbicide would proportionally affect hypocotyls and

195   radicles (Figure 1). It was observed that at a dose of 0 %, the radicle length was about 2/3 of

196   the seedling length. But, in the doses of 0.06 and 0.12 %, the seedlings presented hypocotyls

197   in greater proportion. There was an inversion in the seedling architecture after the treatment

198   with glyphosate. Heinz; Viegas Neto and Valente (2011) also noticed a reduction in the

199   radicle length. In our study, the hypocotyl becomes larger than the primary root when the

200   seedlings were treated with the herbicide. In the Chinese cabbage seed, tetracycline, whose

201   mechanism of action occurs at the ribosomes level, blocked the mobilization of protein bodies

202   in cotyledons during radicle elongation (LUO et al., 2020). We believe that the explanation is

203   plausible, since glyphosate affects protein synthesis by blocking the EPSPS enzyme

204   (NAKATANI et al., 2014).

205   For these results, it is possible to assume that the development of the aerial part would

206   become a priority during the development with glyphosate. This result is interesting,

207   considering that was observed in other studies that the seed lots with contrasting physiological

208   quality would have no difference for dry matter weight in the hypocotyl, but for radicle

209   (OLIVEIRA et al., 2020). Thus, other metabolic pathways can be activated in these

210   conditions.

211   To better investigate this observation, the average length of the hypocotyl (ALH) and

212   radicle (ALR) in herbicide treatments (0.06 and 0.12 %) were divided by the respective values

213   found in the control treatment (0 %), and the results obtained in percentages.

214   About the hypocotyl, the rates of reductions in the genotypes Emgopa315, UFUS7415,

215   P98Y11, and TMG1264 were 68, 53, 25, and 25%, respectively, in the 0.06% treatment. In

216   the 0.12% treatment, these rates increase to 76, 82, 44, and 49%, respectively. We observe

217   that the reductions were more intense for glyphosate-sensitive cultivars. The herbicide has an

218   inhibitory effect on the organogenesis of sensitive seedlings (BERVALD et al., 2010).

219   In the case of radicles, unlike the hypocotyl, all genotypes were similarly affected,

220   both in the treatment of 0.06% and 0.12%, reinforcing the hypothesis that glyphosate affects

221   radicle development more than the hypocotyl, also seen in soybean by Tillmann and West

222   (2004) and Heinz et al. (2011), and in pea by Mondal et al. (2017).

223   These results show that the radicles are harmed by the herbicide in a drastic way,
224   while the hypocotyl becomes a priority in seedling development. However, even though the
225   hypocotyl has priority, sensitive cultivars do not develop it well, because of EPSPS inhibition.

226   **Reserve mobilization**

227   The weights of the hypocotyl (W.1H), radicle (W.1R), and seedling (W.1Sd) of the
228   genotypes were reduced due of the herbicide treatments (Table 3). The glyphosate-tolerant
229   genotypes showed more dry matter than the glyphosate-sensitive genotypes. This is an
230   interesting result because glyphosate-sensitive and glyphosate-tolerant cultivars could not be
231   differentiated by ALR, but by W.1R; this was possible because the roots of tolerant seedlings
232   are heavier (TORRES et al., 2003). In fact, the average root length does not include secondary
233   roots, developed only by herbicide-tolerant cultivars, and absent or incipient in sensitive
234   genotypes (FUNGUETTO et al., 2004; MELO; FAGIOLI; SÁ, 2013; PÁDUA et al., 2012).

Table 3. Dry matter weigh of hypocotyl, radicle and seedling of four soybean cultivars treated with three (0, 0.06, and 0.12 %) doses of glyphosate.

Dry matter weigh of hypocotyl – P.1H (μg)			
Dosage	Emgopa 315	UFUS 7415	P98Y11	TMG 1264
0.00 %	14.40 Ba	25.30 Aa	26.7 Aa	27.9 Aa
0.06 %	7.23 Cb	15.40 Bb	21.5 Ab	23.1 Ab
0.12 %	5.14 Cc	8.85 Bc	18.8 Ac	17.8 Ac
Mean	17.7			
CV (%)	12.4			
Dry matter weigh of radicle – P.1R (μg)			
Dosage	Emgopa 315	UFUS 7415	P98Y11	TMG 1264
0.00 %	4.70 Ca	10.80 Aa	9.56 Ba	10.70 Aa
0.06 %	1.95 Cb	4.04 Bb	5.33 Ab	6.16 Ab
0.12 %	1.68 Db	3.47 Cb	4.82 Bb	5.91 Ab
Mean	5.8			
CV (%)	20.3			
Dry matter weigh of seedling – P.2C (μg)			
Dosage	Emgopa 315	UFUS 7415	P98Y11	TMG 1264
0.00 %	19.10 Ba	36.1 Aa	36.2 Aa	38.6 Aa
0.06 %	9.18 Cb	19.4 Bb	26.8 Ab	29.2 Ab
0.12 %	6.82 Cb	12.3 Bc	23.6 Ac	23.7 Ac
Mean	23.4			
CV (%)	11.8			

* Averages followed by the same letter, uppercase in the horizontal and lowercase in the vertical, do not differ, at the level of 5 % probability.

235

236   Regarding the contrast between the dry matter contained in the seeds and the dry
237   matter of the seedlings, it was possible to estimate the percentage of dry matter mobilized for
238   the hypocotyls, roots, and seedlings (Table 4).

239

Table 4. Mobilization of reserves for hypocotyl, radicle and seedling, and Efficiency in converting mobilized reserves (ECMR) of soybean cultivars treated with three (0, 0.06 and 0.12 %) doses of glyphosate.

Mobilized Seed Reserve - RSM (%)				
Dosage	Emgopa 315	UFUS 7415	P98Y11	TMG 1264
0.00 %	38.9 Ca	43.6 Ba	34.5 Da	50.3 Aa
0.06 %	25.9 Bb	26.6 Bb	29.6 Bb	40.1 Ab
0.12 %	22.3 Cb	23.1 Cb	27.4 Bb	33.0 Ac
Mean	32.95			
CV (%)	19.01			
Mobilized reserve into seedling – MRS (%)				
Dosage	Emgopa 315	UFUS 7415	P98Y11	TMG 1264
0.00 %	39.9 Ba	60.5 Aa	57.1 Aa	62.8 Aa
0.06 %	31.3 Cb	55.3 Aa	49.1 Bb	58.1 Aa
0.12 %	27.2 Db	37.9 Cb	46.1 Bb	56.0 Aa
Mean	48.44			
CV(%)	19.10			
Mobilized reserve into hypocotyl – MRH (%)				
Dosage	Emgopa 315	UFUS 7415	P98Y11	TMG 1264
0.00 %	30.0 Ba	42.5 Aa	42.1 Aa	45.4 Aa
0.06 %	25.0 Bb	43.7 Aa	39.3 Aa	45.8 Aa
0.12 %	20.6 Cb	27.1 Bb	36.7 Aa	42.0 Aa
Mean	36.69			
CV (%)	20.21			
Mobilized reserve into radicle – RMR (%)				
Dosage	Emgopa 315	UFUS 7415	P98Y11	TMG 1264
0.00 %	9.88 Ca	17.9 Aa	15.0 Ba	17.4 Aa
0.06 %	6.51 Bb	11.6 Ab	9.79 Ab	12.3 Ab
0.12 %	6.62 Cb	10.8 Bb	9.41 Bb	14.0 Ab
Mean	11.76			
CV(%)	20.45			
Efficiency in converting mobilized reserve (ECMR)				
%	Emgopa 315	UFUS7415	P98Y11	TMG 1264
0.00 %	14.8 Da	26.2 Ba	19.5 Ca	31.6 Aa
0.06 %	7.59 Cb	14.5 Bb	14.5 Bb	23.3 Ab
0.12 %	5.78 Dc	8.61 Cc	12.5 Bc	18.4 Ac
Mean	16.44			
CV (%)	11.90			

* Averages followed by the same letter, uppercase in the horizontal and lowercase in the vertical, do not differ, at the level of 5 % probability.

240

241   First, about the hypocotyl, it was noted that treatments with the herbicide reduced the
242 reserve rate mobilized in cultivars herbicide-sensitive, Emgopa315 (treatments 0.06 and
243 0.12%) and UFUS7415 (treatment 0.12%); however, for the tolerant genotypes (P98Y11 and
244 TMG1264), there was no reduction in the mobilized reserve due to the herbicide. Given these
245 results, the development of the hypocotyl, in fact, is different for cultivars sensitive and
246 tolerant to glyphosate (HEINZ; VIEGAS NETO; VALENTE, 2011; TILLMANN; WEST,
247 2004).

248   For the radicle, regardless of the genotype and the concentration of the herbicide, the
249 mobilization of reserves was reduced, which corroborates the observation that the radicles of

250   the four genotypes are affected equally. Therefore, in the case of the radicle, which is more

251   affected than the hypocotyl in treatment with the herbicide (TILLMANN; WEST, 2004),

252   neither the length nor the weight or the reserve mobilization efficiently differentiated the

253   cultivars in terms of tolerance or sensitivity to the herbicide. As presented by Torres et al.

254   (2003), we also consider root development as a marker feature to differentiate genotypes in

255   relation to glyphosate tolerance, especially with regard to the development of secondary roots.

256   In the case of seedlings, the effects varied. There were differences between classes of

257   genotypes, herbicide-tolerant and herbicide-sensitive, as well as between sensitive genotypes

258   (Emgopa315 and UFUS7415) and between tolerant genotypes (P98Y11 and TMG1264).

259   Except for TMG1264, the treatments with the herbicide (0.06 and/or 0.12 %) reduced the

260   reserve mobilized into the seedlings. Possibly, it is a result associated with the better quality

261   of the seed lot (ANDRADE; COELHO; PADILHA, 2019). Anyway, the accumulation of dry

262   matter in seedlings from stressed seeds is less than that of non-stressed seeds (SILVA;

263   AZEVEDO NETO; GHEYI, 2019).

264   **Efficiency of Conversion of Seed Reserve to Seedling**

265   Regarding the percentage of mobilized reserves of the cotyledons, the herbicide

266   affected all genotypes at different levels (Table 4). Notably, the glyphosate-tolerant genotype

267   and with better physiological quality, TMG1264, was the one that most mobilized reserves of

268   its cotyledons and presented the greatest efficiency in converting seed reserves into seedlings.

269   Further studies should assess whether this is a particularity of the genotype or an issue of

270   initial physiological quality, but there are literatures reporting the better quality lots result in

271   seedlings with more dry matter (ANDRADE; COELHO; PADILHA, 2019; OLIVEIRA et al.,

272   2020). Anyway, a focused investigation on the subject is necessary, because if there is an

273   important genetic factor controlling this trait, genetic improvement can be applied.

274   Notable characteristics of the bioassay in this study were the reduction in the length

275   and thickening of the hypocotyl-radicle axis of the seedlings. To verify this, the linear weights

276   (ug / cm) of the hypocotyl (LWH), root (LWR) and seedling (LWS) were estimated (Table 5).

277   We observed that the genotypes increased their linear weights due to treatments with

278   herbicides. Under these conditions, the seedlings concentrate more reserves, thickening the

279   hypocotyl-radicle axis, as also seen by Funguetto et al. (2004). This result corroborates with

280   the herbicide effect in reducing the seedlings length. Considering that both the seedlings

281   length and the dry matter weight of the seedlings were affected by treatments with the

282   herbicide, the linear weight showed that the length was more affected than the weight since

283   the linear weight increased due to the herbicide concentration in all treatments.

Table 5. Hypocotyl, radicle and seedling linear weigh of four soybean cultivars treated with three (0, 0.06 and 0.12 %) doses of glyphosate.

Linear weigh of the hypocotyl – LWH ($\mu g.cm^{-1}$)			
Dosage	Emgopa 315	UFUS7415	P98Y11	TMG 1264
0.00 %	2.90 Bb	3.24 Bc	4.11 Ab	3.72 Ab
0.06 %	4.52 Aa	4.44 Ab	4.31 Ab	4.08 Ab
0.12 %	4.20 Ba	6.26 Aa	4.93 Ba	4.64 Ba
Mean	4.28			
CV (%)	16.8			
Linear weigh of the radicle – LWR ($\mu g.cm^{-1}$)			
Dosage	Emgopa 315	UFUS7415	P98Y11	TMG 1264
0.00 %	0.37 Cb	1.72 Ab	0.88 Bc	0.79 Bc
0.06 %	1.04 Ca	3.63 Aa	2.34 Bb	2.04 Bb
0.12 %	0.96 Ca	3.24 Aa	2.89 Ba	2.56 Ba
Mean	1.87			
CV (%)	33.3			
Linear weigh of the seedling – LWS ($\mu g.cm^{-1}$)			
Dosage	Emgopa 315	UFUS7415	P98Y11	TMG 1264
0.00 %	1.08 Cb	2.55 Ac	2.08 Bc	1.84 Bc
0.06 %	2.60 Ca	4.17 Ab	3.67 Bb	3.37 Bb
0.12 %	2.30 Da	4.92 Aa	4.30 Ba	3.85 Ca
Mean	3.06			
CV (%)	14.5			

* Averages followed by the same letter, uppercase in the horizontal and lowercase in the vertical, do not differ, at the level of 5 % probability.

284

285   It was possible to observe that some responses of the genotypes vary in relation to the

286   herbicide, while others are common to all. In fact, herbicide-tolerant cultivars form normal

287   seedlings under treatment with glyphosate but show sensitivity at certain concentrations.

288   Length, weight and mobilization of reserves are fundamental characteristics of the initial

289   phase of seedling development and can be considered in breeding programs. The

290   identification of QTLs associated with the use of seed reserves in selected RILs (CHENG et

291   al., 2013), for example, provided a glimpse into the use of marker-assisted selection to

292   improve the mobilization of rice reserves.

293   **Conclusions**

294   1. The herbicide affects the length, weight, and reserves mobilization of the evaluated

295   genotypes, but in a more striking way those that are glyphosate-sensitive.

296   2. During germination, the herbicide affects the radicle more than the seedling hypocotyl,

297   making it proportionally larger than the radicle, changing the seedling morphology.

298   3. The mobilization of reserves discriminates genotypes regarding tolerance to the herbicide,

299   reinforcing and complementing the seedlings length analyzes.

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