Seed reserve mobilization and seedling morphology in a bioassay for the
detection of genetically modified soybean

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

Keywords: dynamics of seed reserve, Glycine max, herbicide.

Introduction

The germination test (BRASIL, 2009) is one of the first analyses carried out on a seed
lot and is performed to assess the germinative potential of the seeds. However, the
germination rate observed in the test does not guarantee the establishment of a crop,
especially in adverse environmental situations, when the mobilization of seed reserves for
seedlings is not satisfactory (OLIVEIRA et al., 2020). In this respect, high vigor seeds are
desired by producers because they emerge faster and more uniformly from the soil,
accumulate more dry mass in the plants, which are also higher (HENNING et al., 2010).

The initial stages of the seedling development coincide with the period of crop
establishment, moment of great apprehension of farmers, aware of the relationship between
the formation of the stand and high yield (HENNING et al., 2010). Well-consolidated stand
increases soil cover, reduces evaporation of available water, reflecting positively on
productivity, improving the competitive power of crops against weeds (DIAS et al., 2011).

According to Mohammadi et al. (2011), the initial weight of the seed, the mobilized
fraction of reserves, and the efficiency in converting these reserves to dry matter are relevant
components to be evaluated during the heterotrophic growth of the seedlings. During this
stage, it is very important to observe how and how much of the seed reserves are mobilized
under favorable or unfavorable scenarios for a crop. Studies showed that more vigorous seeds have increased their capacity to mobilize reserves, and consequently, a better initial performance (HENNING et al., 2010; OLIVEIRA et al., 2020).

Crops face adversities from planting to harvest, with germination and seedling emergence being considered the most critical and potentially most sensitive stages to environmental stresses (ALI et al., 2018). Many examples may be presented. In wheat, the seedlings length, the weight of the mobilized reserve, and the fraction of use of this reserve become reduced under water and salt stress (SOLTANI; Gholipoor; Zeinali, 2006). In soybeans, under water stress, the seedling is impaired, being the development of the hypocotyl is more affected than the radicle (PEREIRA; PEREIRA; DIAS, 2013). The use of desiccants in crops can affect the germination and vigor of harvested soybean seeds, with a negative influence on the mobilization of reserves (DELGADO; COELHO; BUBA, 2015).

Copper causes negative effects on cell elongation and cell division in Vicia sativa roots and inhibits the mobilization of proteins from cotyledons (MUCCIFORA; BELLANI, 2013). In general, the effects are proportional to the intensity of the stress, which increases the consumption of reserves, without this meaning conversion into dry seedling mass (OLIVEIRA et al., 2020). On the other hand, the pretreatment of soybean seeds with 'cold plasma' improved the germination rate, length and dry weight of the seedlings, as well as the mobilization of seed reserves and efficiency in the use of reserves (LING et al., 2014).

Projects on the mobilization of reserves contribute to the understanding of the potential for acclimatization of seedlings (SILVA; AZEVEDO NETO; GHEYI, 2019). Expanding knowledge about the process of mobilizing seed reserves, under the most diverse contexts, can contribute to a better understanding of this important stage. Our research group realized that the availability of genetically modified cultivars for glyphosate tolerance is a good model for studies on the effects of herbicides on the initial development of seedlings. Considered as a model species, our research indeed brings relevant information for soybean and other crops species.

Given the above, the objective of this work was to study the dynamics of soybean seed reserves during the heterotrophic development of the seedlings and their morphology, in an artificial environment simulating the physiological stress that soybean seeds face during germination.

**Materials and Methods**

**Water content**
To measure the water content (WC) of the studied seed lots, three replicates of 50 seeds (W1) were weighed, taken to the oven at 105 °C for 24 hours, and weighed again (W2) on a scale with four decimal places. Based on these data, the following formula was applied (SOLTANI; GHOLOPOOR; ZEINALI, 2006).

\[
WC = \left(\frac{W2 - W1}{W1}\right) * 100
\]

**Germination test**

The germination test was carried out according to the recommendations of the Regulation for Seed Analysis (BRASIL, 2009), with adaptations (PEREIRA et al., 2009a), to obtain the first count and the final germination count. Four soybean cultivars were analyzed, 2 sensitive (BRS511, EMGOPA315) and 2 glyphosate-tolerant (TMG1264, P98Y11). The samples were submitted to germination in substrate moistened with water in the control treatment and solutions of the herbicide (0.06 and 0.12%) in the other treatments (PEREIRA et al., 2009a), in the proportion of 2.5 times the weight of dry paper (BRASIL, 2009). A completely randomized design was adopted, in a factorial scheme, composed of 4 genotypes x 3 doses of the herbicide, with 4 replications.

**Seedling length test**

The seedling length test was carried out according to (PEREIRA et al., 2009b), with adaptations regarding the moistening of the substrate, with three solutions of the herbicide (0, 0.06 and 0.12% e. a.). A completely randomized design was adopted, in a factorial scheme, 3 cultivars x 3 doses of the herbicide, with 10 repetitions, with each experimental plot consisting of 10 seeds. After the rolls confection, they were packed in plastic bags, with the open end for ventilation, and kept in BOD for 7 days at 25 °C. At the end of the test, the average lengths of seedling (ALS), hypocotyl (ALH) and radicle (ALR) of each experimental plot were obtained, using a millimeter ruler.

From the average lengths, the proportions of hypocotyl and root of the seedlings in each treatment were known, using the following equations:

\[
\text{Hypocotyl Proportion (\% H / S)} = \frac{ALH}{ALS} \times 100
\]

\[
\text{Radicle Proportion (\% R / S)} = \frac{ALR}{ALS} \times 100
\]

Additionally, the percentages of reduction observed for hypocotyl (RDH) and radicle (RDR) were obtained, due the treatments. For this, the average lengths of the hypocotyl and radicle, under treatment with the herbicide, were compared with those of the experimental control. The following equations were used:

\[
\text{Hypocotyl reduction [0 to 0.06\%]} = \left[1 - \frac{ALH}{ALH 0\%}\right] * 100
\]

\[
\text{Radicle reduction [0 to 0.06\%]} = \left[1 - \frac{ALR}{ALR 0\%}\right] * 100
\]
Hypocotyl reduction [0 to 0.12%] = \[1 - (0.12\% \text{ ALH} / 0\% \text{ ALH})\] * 100

Radicle reduction [0 to 0.12%] = \[1 - (\text{ALR 0.12\%} / \text{ALR 0\%})\] * 100

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

Previously, the 120 experimental plots (10 seeds each) separated for the seedling length test were weighed on a precision scale, providing the weight of 10 seeds (W.10) in micrograms. As the water content of each seed lot was previously obtained, it supported the estimation of the dry reserve weight (DW.10) in each experimental plot or in each seed (W.1S) for mobilization from the seeds to seedlings during germination (PEREIRA; PEREIRA; DIAS, 2015).

After measuring the seedlings lengths, the cotyledons, hypocotyls, and radicles of each experimental plot were separated with a stylus and collected in their respective envelopes. Then, these envelopes were kept in an oven at a regulated temperature of 80 °C for 24 hours. From that period, the contents of the envelopes were weighed and the data, in micrograms (\(\mu g\)), used to analyze the mobilization of reserves (PEREIRA; PEREIRA; DIAS, 2015).

Considering the number of seedlings evaluated, the average weight of the dry matter of the hypocotyl (W.1H), radicle (W.1R), seedling (W.1Sd), and cotyledon pair (W.2C) of each experimental plot was obtained. From this information, the first aspect analyzed was the rate of reduction of the seed reserve (% SRR). Given the prior knowledge of the dry weight of the seed (W.1S) and the dry weight of the pair of cotyledons (W.1PC), it was possible to quantify the amount of seed mobilized reserve.

\[\%\text{SRR} = (W.1S - W.1PC) / W.1S.\]

From the knowledge of the dry weight of hypocotyl (W.1H), radicle (W.1R), and seedling (W.1Sd), as well as the reserve that was mobilized from the seeds (SRR = W.1S – W.1PC), it was possible to calculate the proportion of reserve mobilized to the hypocotyl (RMH), radicle (RMR) and seedling (RMS).

\[\text{RMH} = (W.1H / SRR) \times 100\]
\[\text{RMR} = (W.1R / SRR) \times 100\]
\[\text{RMS} = (W.1Sd / SRR) \times 100\]

Additionally, we obtained information about the Efficiency of the Conversion of Reserve into Seedling (ERCS). Unlike the variable mobilized reserve for seedling (%SRR), which considers how much of the mobilized reserve has become a seedling, the ECRS variable considers how much of the reserve contained in the seed has become a seedling.

\[\text{ECRS} = (W.1Sd / W.1S) \times 100\]
Finally, from the lengths and weights of the seedlings, it was possible to obtain the linear weights of hypocotyl (LWH), radicle (LWR) and seedling (LWS) in μg / cm.

\[
\begin{align*}
LWH &= \frac{W \cdot 1H}{ALH} \\
LWR &= \frac{W \cdot 1R}{ALR} \\
LWS &= \frac{W \cdot 1Sd}{ALS}
\end{align*}
\]

**Experimental design and data analysis**

The tests were performed in a completely randomized design, in a factorial scheme. The number of repetitions and the size of the experimental plots varied between experiments. The data were subjected to analysis of variance and means compared by the Scott-Knott test, at the level of 5% probability.

**Results and Discussion**

**Germination test**

Analyzing the first and the final counts of germination data (Table 1), it was notable that both herbicide solutions (0.06, and 0.12 %) inhibited the development of normal seedlings by the genotypes, like observed also by Bervald et al. (2010) and Pereira et al., (2018). In the case of glyphosate-tolerant cultivars, the highest concentration of the herbicide (0.12 %) affected the first and the final counts of germination, as observed by Pereira et al., (2018), however, the glyphosate-tolerant seedlings developed secondary roots, while the sensitive ones did not (Figure 1). The absence of secondary roots in glyphosate-sensitive seedlings is a striking feature of this bioassay (BERVALD et al., 2010; MELO; FAGIOLI; SÁ, 2013; PÁDUA et al., 2012). According to Albrecht et al. (2014), the physiological quality and the vigor of the lots fall due to the concentration of the herbicide.

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Emgopa 315</th>
<th>UFUS 7415</th>
<th>P98Y11 1264</th>
<th>TMG 1264</th>
<th>Emgopa 315</th>
<th>UFUS 7415</th>
<th>P98Y11 1264</th>
<th>TMG 1264</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 %</td>
<td>76.5 Ba</td>
<td>52.0 Ca</td>
<td>54.0 Ca</td>
<td>91.5 Aa</td>
<td>78.0 Ba</td>
<td>56.5 Ca</td>
<td>59.5 Ca</td>
<td>94.0 Aa</td>
</tr>
<tr>
<td>0.06 %</td>
<td>0.5 Cb</td>
<td>0.0 Cb</td>
<td>58.0 Ba</td>
<td>80.5 Ab</td>
<td>1.5 Cb</td>
<td>0.1 Cb</td>
<td>60.5 Ba</td>
<td>94.0 Ab</td>
</tr>
<tr>
<td>0.12 %</td>
<td>0.0 Bb</td>
<td>0.0 Bb</td>
<td>5.5 Bb</td>
<td>62.0 Ac</td>
<td>0.5 Cb</td>
<td>0.1 Cb</td>
<td>27.5 Bb</td>
<td>78.0 Ab</td>
</tr>
<tr>
<td>Mean</td>
<td>37.4</td>
<td>45.4</td>
<td></td>
<td></td>
<td>26.7</td>
<td>20.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 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.
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 toothpeaks moistened with glyphosate to differentiate tolerant and non-tolerant soybean cultivars, Silva et al. (2015) stated that the...
injured plants of transgenic cultivars kept growing the hypocotyl (main stem) and
developed new lateral branches instead of new lateral radicles like in our research. This
difference may be explained because their role plants were not exposed to glyphosate at the
initial growing stages, then the glyphosate followed the xylem flux to the aerial parts of the
seedlings. Differently, our seedlings were completely exposed to the glyphosate solution
treatments, then we can affirm the radicule tissues are more sensitive to glyphosate, since no
secondary branches were observed in our results.

Seedling length test

The herbicide treatments reduced the lengths of the hypocotyl (ALH), radicle (ALR),
and seedling (ALS) (Table 2). In the herbicide treatments, the secondary roots are absent or
incipient in sensitive seedlings, with an aspect of blocked development. Cultivars that carry
the CP4 EPSPS gene are glyphosate-tolerant (NAKATANI et al., 2014). When these cultivars
are submitted to germination in substrate moistened with glyphosate solutions, secondary
roots are present, but in length, number, and volume smaller than in the control treatment, as
observed by Pereira et al., (2018); in fact, the herbicide affects the development of sensitive
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.

<table>
<thead>
<tr>
<th></th>
<th>Emgopa 315</th>
<th>UFUS 7415</th>
<th>P98Y11</th>
<th>TMG 1264</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Average length of the hypocotyl - ALH (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00 %</td>
<td>5.12 Ca</td>
<td>7.89 Aa</td>
<td>6.79 Ba</td>
<td>7.54 Aa</td>
</tr>
<tr>
<td>0.06 %</td>
<td>1.66 Cb</td>
<td>3.65 Bb</td>
<td>5.09 Ab</td>
<td>5.67 Ab</td>
</tr>
<tr>
<td>0.12 %</td>
<td>1.22 Bb</td>
<td>1.42 Bc</td>
<td>3.83 Ac</td>
<td>3.87 Ac</td>
</tr>
<tr>
<td>Mean</td>
<td>4.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td>18.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Emgopa 315</th>
<th>UFUS 7415</th>
<th>P98Y11</th>
<th>TMG 1264</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Average length of the radicle - ALR (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00 %</td>
<td>13.2 Aa</td>
<td>6.48 Ca</td>
<td>11.3 Ba</td>
<td>13.5 Aa</td>
</tr>
<tr>
<td>0.06 %</td>
<td>1.91 Bb</td>
<td>1.15 Bb</td>
<td>2.28 Ab</td>
<td>3.03 Ab</td>
</tr>
<tr>
<td>0.12 %</td>
<td>1.74 Ab</td>
<td>1.08 Ab</td>
<td>1.68 Ab</td>
<td>2.33 Ab</td>
</tr>
<tr>
<td>Mean</td>
<td>4.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td>31.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Emgopa 315</th>
<th>UFUS 7415</th>
<th>P98Y11</th>
<th>TMG 1264</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Average length of the seedling - ALS (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00 %</td>
<td>18.3 Ba</td>
<td>14.4 Ca</td>
<td>18.1 Ba</td>
<td>21.0 Aa</td>
</tr>
<tr>
<td>0.06 %</td>
<td>3.56 Bb</td>
<td>4.80 Bb</td>
<td>7.38 Ab</td>
<td>8.71 Ab</td>
</tr>
<tr>
<td>0.12 %</td>
<td>2.96 Bb</td>
<td>2.50 Bc</td>
<td>5.51 Ac</td>
<td>6.19 Ac</td>
</tr>
<tr>
<td>Mean</td>
<td>9.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td>17.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Averages followed by the same letter, uppercase in the horizontal and lowercase in the vertical, do not
differ, at the level of 5 % probability.
According to Funguetto et al. (2004); Heinz; Viegas Neto and Valente (2011); Pereira et al. 2018; and Tillmann and West (2004) the length reductions are more striking for glyphosate-sensitive genotypes. In our study, this was a notable occurrence.

Once it was verified the average lengths (ALH, ALR, and ALP) are reduced, it was questioned whether treatments with the herbicide would proportionally affect hypocotyls and radicles (Figure 1). It was observed that at a dose of 0 %, the radicle length was about 2/3 of the seedling length. But, in the doses of 0.06 and 0.12 %, the seedlings presented hypocotyls in greater proportion. There was an inversion in the seedling architecture after the treatment with glyphosate. Heinz; Viegas Neto and Valente (2011) also noticed a reduction in the radicle length. In our study, the hypocotyl becomes larger than the primary root when the seedlings were treated with the herbicide. In the Chinese cabbage seed, tetracycline, whose mechanism of action occurs at the ribosomes level, blocked the mobilization of protein bodies in cotyledons during radicle elongation (LUO et al., 2020). We believe that the explanation is plausible, since glyphosate affects protein synthesis by blocking the EPSPS enzyme (NAKATANI et al., 2014).

For these results, it is possible to assume that the development of the aerial part would become a priority during the development with glyphosate. This result is interesting, considering that was observed in other studies that the seed lots with contrasting physiological quality would have no difference for dry matter weight in the hypocotyl, but for radicle (OLIVEIRA et al., 2020). Thus, other metabolic pathways can be activated in these conditions.

To better investigate this observation, the average length of the hypocotyl (ALH) and radicle (ALR) in herbicide treatments (0.06 and 0.12 %) were divided by the respective values found in the control treatment (0 %), and the results obtained in percentages.

About the hypocotyl, the rates of reductions in the genotypes Emgopa315, UFUS7415, P98Y11, and TMG1264 were 68, 53, 25, and 25%, respectively, in the 0.06% treatment. In the 0.12% treatment, these rates increase to 76, 82, 44, and 49%, respectively. We observe that the reductions were more intense for glyphosate-sensitive cultivars. The herbicide has an inhibitory effect on the organogenesis of sensitive seedlings (BERVALD et al., 2010).

In the case of radicles, unlike the hypocotyl, all genotypes were similarly affected, both in the treatment of 0.06% and 0.12%, reinforcing the hypothesis that glyphosate affects radicle development more than the hypocotyl, also seen in soybean by Tillmann and West (2004) and Heinz et al. (2011), and in pea by Mondal et al. (2017).
These results show that the radicles are harmed by the herbicide in a drastic way, while the hypocotyl becomes a priority in seedling development. However, even though the hypocotyl has priority, sensitive cultivars do not develop it well, because of EPSPS inhibition.

**Reserve mobilization**

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

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Emgopa 315</th>
<th>UFUS 7415</th>
<th>P98Y11</th>
<th>TMG 1264</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 %</td>
<td>14.40 Ba</td>
<td>25.30 Aa</td>
<td>26.7 Aa</td>
<td>27.9 Aa</td>
</tr>
<tr>
<td>0.06 %</td>
<td>7.23 Cb</td>
<td>15.40 Bb</td>
<td>21.5 Ab</td>
<td>23.1 Ab</td>
</tr>
<tr>
<td>0.12 %</td>
<td>5.14 Cc</td>
<td>8.85 Bc</td>
<td>18.8 Ac</td>
<td>17.8 Ac</td>
</tr>
<tr>
<td>Mean</td>
<td>17.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td>12.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Dry matter weigh of radicle – P.1R (μg)

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Emgopa 315</th>
<th>UFUS 7415</th>
<th>P98Y11</th>
<th>TMG 1264</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 %</td>
<td>4.70 Ca</td>
<td>10.80 Aa</td>
<td>9.56 Ba</td>
<td>10.70 Aa</td>
</tr>
<tr>
<td>0.06 %</td>
<td>1.95 Cb</td>
<td>4.04 Bb</td>
<td>5.33 Ab</td>
<td>6.16 Ab</td>
</tr>
<tr>
<td>0.12 %</td>
<td>1.68 Db</td>
<td>3.47 Cb</td>
<td>4.82 Bb</td>
<td>5.91 Ab</td>
</tr>
<tr>
<td>Mean</td>
<td>5.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td>20.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Dry matter weigh of seedling – P.2C (μg)

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Emgopa 315</th>
<th>UFUS 7415</th>
<th>P98Y11</th>
<th>TMG 1264</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 %</td>
<td>19.10 Ba</td>
<td>36.1 Aa</td>
<td>36.2 Aa</td>
<td>38.6 Aa</td>
</tr>
<tr>
<td>0.06 %</td>
<td>9.18 Cb</td>
<td>19.4 Bb</td>
<td>26.8 Ab</td>
<td>29.2 Ab</td>
</tr>
<tr>
<td>0.12 %</td>
<td>6.82 Cb</td>
<td>12.3 Bc</td>
<td>23.6 Ac</td>
<td>23.7 Ac</td>
</tr>
<tr>
<td>Mean</td>
<td>23.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td>11.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

Regarding the contrast between the dry matter contained in the seeds and the dry matter of the seedlings, it was possible to estimate the percentage of dry matter mobilized for the hypocotyls, roots, and seedlings (Table 4).
First, about the hypocotyl, it was noted that treatments with the herbicide reduced the reserve rate mobilized in cultivars herbicide-sensitive, Emgopa315 (treatments 0.06 and 0.12%) and UFUS7415 (treatment 0.12%); however, for the tolerant genotypes (P98Y11 and TMG1264), there was no reduction in the mobilized reserve due to the herbicide. Given these results, the development of the hypocotyl, in fact, is different for cultivars sensitive and tolerant to glyphosate (HEINZ; VIEGAS NETO; VALENTE, 2011; TILLMANN; WEST, 2004).

For the radicle, regardless of the genotype and the concentration of the herbicide, the mobilization of reserves was reduced, which corroborates the observation that the radicles of...
the four genotypes are affected equally. Therefore, in the case of the radicle, which is more affected than the hypocotyl in treatment with the herbicide (TILLMANN; WEST, 2004), neither the length nor the weight or the reserve mobilization efficiently differentiated the cultivars in terms of tolerance or sensitivity to the herbicide. As presented by Torres et al. (2003), we also consider root development as a marker feature to differentiate genotypes in relation to glyphosate tolerance, especially with regard to the development of secondary roots.

In the case of seedlings, the effects varied. There were differences between classes of genotypes, herbicide-tolerant and herbicide-sensitive, as well as between sensitive genotypes (Emgopa315 and UFUS7415) and between tolerant genotypes (P98Y11 and TMG1264). Except for TMG1264, the treatments with the herbicide (0.06 and/or 0.12 %) reduced the reserve mobilized into the seedlings. Possibly, it is a result associated with the better quality of the seed lot (ANDRADE; COELHO; PADILHA, 2019). Anyway, the accumulation of dry matter in seedlings from stressed seeds is less than that of non-stressed seeds (SILVA; AZEVEDO NETO; GHEYI, 2019).

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

Regarding the percentage of mobilized reserves of the cotyledons, the herbicide affected all genotypes at different levels (Table 4). Notably, the glyphosate-tolerant genotype and with better physiological quality, TMG1264, was the one that most mobilized reserves of its cotyledons and presented the greatest efficiency in converting seed reserves into seedlings. Further studies should assess whether this is a particularity of the genotype or an issue of initial physiological quality, but there are literatures reporting the better quality lots result in seedlings with more dry matter (ANDRADE; COELHO; PADILHA, 2019; OLIVEIRA et al., 2020). Anyway, a focused investigation on the subject is necessary, because if there is an important genetic factor controlling this trait, genetic improvement can be applied.

Notable characteristics of the bioassay in this study were the reduction in the length and thickening of the hypocotyl-radicle axis of the seedlings. To verify this, the linear weights (ug / cm) of the hypocotyl (LWH), root (LWR) and seedling (LWS) were estimated (Table 5). We observed that the genotypes increased their linear weights due to treatments with herbicides. Under these conditions, the seedlings concentrate more reserves, thickening the hypocotyl-radicle axis, as also seen by Funguetto et al. (2004). This result corroborates with the herbicide effect in reducing the seedlings length. Considering that both the seedlings length and the dry matter weight of the seedlings were affected by treatments with the herbicide, the linear weight showed that the length was more affected than the weight since the linear weight increased due to the herbicide concentration in all treatments.
It was possible to observe that some responses of the genotypes vary in relation to the herbicide, while others are common to all. In fact, herbicide-tolerant cultivars form normal seedlings under treatment with glyphosate but show sensitivity at certain concentrations. Length, weight and mobilization of reserves are fundamental characteristics of the initial phase of seedling development and can be considered in breeding programs. The identification of QTLs associated with the use of seed reserves in selected RILs (CHENG et al., 2013), for example, provided a glimpse into the use of marker-assisted selection to improve the mobilization of rice reserves.

**Conclusions**

1. The herbicide affects the length, weight, and reserves mobilization of the evaluated genotypes, but in a more striking way those that are glyphosate-sensitive.
2. During germination, the herbicide affects the radicle more than the seedling hypocotyl, making it proportionally larger than the radicle, changing the seedling morphology.
3. The mobilization of reserves discriminates genotypes regarding tolerance to the herbicide, reinforcing and complementing the seedlings length analyzes.

**Acknowledgements**
The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for providing the scholarships to the authors FCLC and SLP.

References


ALI, Q. et al. Assessment of drought tolerance in mung bean cultivars/lines as depicted by the activities of germination enzymes, seedling’s antioxidative potential and nutrient acquisition. *Archives of Agronomy and Soil Science*, v. 64, n. 1, p. 84–102, 2 jan. 2018. DOI: 10.1080/03650340.2017.1335393


FUNGUETTO, C. I. et al. Detecção de sementes de soja geneticamente modificada tolerante


NAKATANI, A. S. et al. Effects of the glyphosate-resistance gene and of herbicides applied...
to the soybean crop on soil microbial biomass and enzymes. *Field Crops Research*, v. 162, p. 20–29, 1 jun. 2014. DOI: 10.1016/j.fcr.2014.03.010


