

# Physiological quality and its relation to the maturity stages of maize seeds

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## ABSTRACT

**Objective:** To determine the physiological quality of maize seeds from the F6 (Yellow×V-54A) experimental variety and its relation to the maturation stages of the seeds, as well as its association with physiological maturity.

**Methodology:** Eight cob samples were assessed to determine the presence of milk line and black layer, moisture content, and dry matter accumulation during seed maturation. A germination test was used to establish physiological quality. The experiment included a completely randomized design with replicated measures at 5% significance.

**Results:** The milk line decreased (100 to 10%) and the black layer gradually appeared (0 to 99%) as seeds matured. Moisture content decreased (58.1 to 25.4%) as sampling progressed, resulting from the highest biomass accumulation (74.6%) and physiological quality (72.5%). The percentage of ungerminated seeds reached 94.3% when seeds were immature; however, this percentage decreased to 18.5% at physiological maturity. Seedling abnormalities ranged from 3.8 to 16.8%.

**Conclusions:** The physiological quality of maize seeds depended on their physical attributes rather than on their physiological maturity.

**Keywords:** *Zea mays* L., milk line, black layer, physiological maturity.

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## INTRODUCTION

Seed production largely depends on the environmental conditions that take place during the growth and development of the crops (Miya *et al.*, 2017). Some indicators of the physiological maturity of maize include moisture content, maximum dry matter accumulation, and presence of black layer. These parameters are used to determine the quality of the seeds (Ferreira *et al.*, 2013; Sripathy and Groot, 2023).

The maximum accumulation of dry matter takes place when the seed reaches its physiological maturity ( $\approx 35\%$  moisture) (Tadeo-Robledo *et al.*, 2010). Subsequently, several physiological processes take place, depending on environmental conditions.



These processes contribute to the deterioration of the seed. In this regard, Jacob *et al.* (2014) suggested that crops must be harvested as soon as possible after they reach their physiological maturity. Some morpho-physiological changes have been identified as indicators of physiological maturity: the presence of the black layer, the disappearance of the milk line, and the decrease of moisture content (Estrada-Urbina *et al.*, 2023a). Meanwhile, Bewley *et al.* (2013) and Dayal *et al.* (2014) indicate that harvest maturity occurs in a 7-10-day interval after the seeds have reached physiological maturity (<20% moisture content). Nevertheless, these characteristics depend on genotype and sowing date.

In other studies, the seed quality is related to their maturity. Consequently, the highest germination rate takes place when the seeds have a high moisture content, before they reach physiological maturity (Carvajal *et al.*, 2017; Mancera and Ramírez, 2018). In addition, the maximum seed quality potential is recorded when they accumulate the highest dry matter percentage and the lowest moisture level (Lozano-Pérez, 2021). Therefore, the objective of this study was to determine the physiological quality of maize seeds from the F6 (Yellow×V-54A) experimental variety and its relation to the maturation stages of the seeds, as well as its association with the morphological markers related to physiological maturity.

## **MATERIALS AND METHODS**

### **Location of the experiment and plant material**

The study was carried out in the Análisis de Semillas lab of the Colegio de Postgraduados - Campus Montecillo (19° 27' 54.7" N and 98° 54' 24.3" W). The F6 (Yellow×V-54A) experimental maize (*Zea mays* L.) variety was used in the experiment. This variety was grown and harvested during the spring-summer season (year 2022), in experimental fields of the Colegio de Postgraduados.

### **Seed sampling**

Eight samples were taken from five cobs. The first was at 20 days after flowering (daf) —*i.e.*, 106 days after sowing (das). Afterwards, the samples were collected in a seven-day interval until the seeds reached physiological maturity (PM). In order to guarantee the highest possible homogeneity and to prevent damages, harvesting was carried out only in phenotypically identical plants.

### **Milk line and black layer**

For each sampling, lengthwise sections were used to visually determine the presence of the milk line (ML) and the black layer (BL) in 100 seeds (Estrada-Urbina *et al.*, 2023a). In addition, the presence of the black layer was registered in eight repetitions of 25 seeds. PM was taken into account when 100% of the seeds had BL (Molina *et al.*, 2003).

### **Moisture content and dry matter**

Moisture content (MC) was determined according to the methodology of the International Seed Testing Association (ISTA, 2021a), by drying two replicates of 5 g of maize at  $130 \pm 2$  °C for 4h. Afterwards, MC was calculated with equation 1.

$$MC = \frac{(IWS - WDS)}{(IWS - WT)} * 100 \quad \text{Equation 1}$$

Where  $MC$ =moisture content (%);  $WT$ =weight of the metal tray (g);  $IWS$ =initial weight of the seed before the drying process (g);  $WDS$ =weight of the dried seed in the metal tray (g).

Seed dry matter (DM) was determined using a similar method than the one used for MC. The results were expressed as accumulated biomass percentages and were calculated with equation 2.

$$DMS = \frac{(WDS * 100)}{(IWS)} \quad \text{Equation 2}$$

Where:  $DMS$ =dry matter of the seed (%);  $IWS$ =initial weight of the seed in the metal tray before the drying process (g);  $WDS$ =weight of the dried seed in the metal tray (g).

### Physiological quality

Physiological quality was determined with the standard germination test (GER) in eight replications of 25 seeds, which were placed in Anchor<sup>®</sup> paper towels, previously soaked in distilled water. Subsequently, the paper towels were rolled up and placed inside a germination chamber at  $25 \pm 1$  °C for 7 days, with a 24 h white light photoperiod. After the incubation period, the seedlings were evaluated based on the criteria of the ISTA (2021b) regarding the number of normal (NNS) and abnormal seedlings (NAS), as well as ungerminated seeds (NUS). The following equations were used to determine physiological quality:

Equation 3 was used to determine the germination percentage.

$$GER = \frac{NNS}{25} * 100 \quad \text{Equation 3}$$

Where:  $GER$ =germination percentage and  $NNS$ =number of normal seedlings.

Equation 4 was used to calculate the percentage of abnormal seedlings.

$$ABNOR = \frac{NAS}{25} * 100 \quad \text{Equation 4}$$

Where:  $ABNOR$ =percentage of abnormal seedlings and  $NAS$ =number of abnormal seedlings.

Equation 5 was used to establish the percentage of ungerminated seeds.

$$NOGS = \frac{NUS}{25} * 100 \quad \text{Equation 5}$$

Where:  $NOGS$ =percentage of non-germinated seeds and  $NUS$ =number of ungerminated seeds.

### Experimental design and statistical analysis

A complete randomized design with repeated measures for the sampling factor was used for the experiment. The analyses of variance (ANOVA) were conducted at an  $\alpha=0.05$  significance with the first-order autoregressive matrix covariance, in order to obtain the lowest value of BIC (Bayesian Information Criterion). ANOVA were carried out with the following statistical model,

$$y_{ij} = \mu + Mue_i + \varepsilon_{ij}$$

where:  $y_{ij}$ = $i$ -th sampling response, in the  $j$ -th replication;  $\mu$ =overall mean;  $Mue_i$ =fixed effect of the  $i$ -th sampling;  $\varepsilon_{ij}$ =experimental error.

The data in percentage were transformed using the  $\arcsen\sqrt{(y/100)}$  equation, to homogenize the variances for their subsequent analysis. When the ANOVA showed statistical differences, the Tukey's test ( $\alpha=0.05$ ) was employed.

All data were analyzed in RStudio software (R Core Team, 2022).

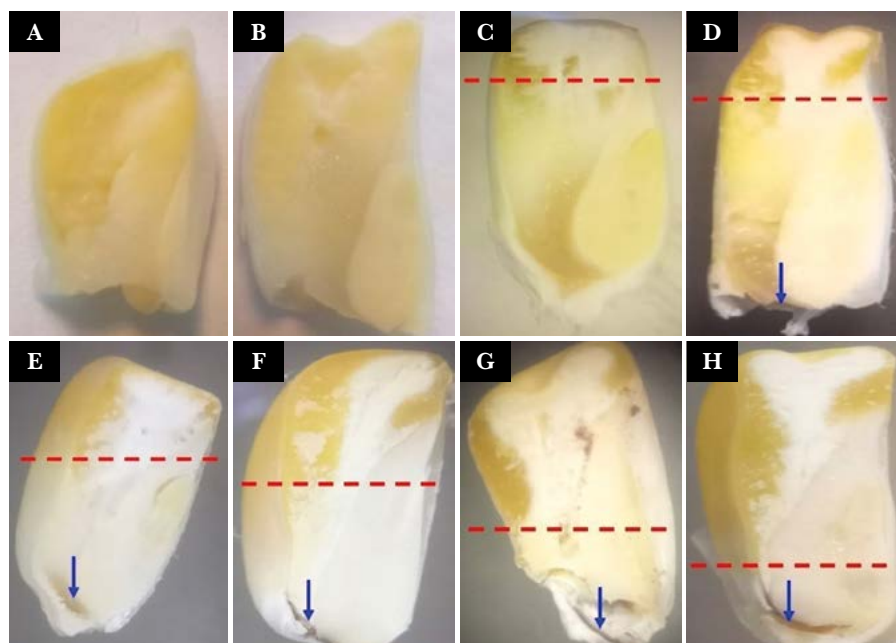
## RESULTS AND DISCUSSION

### Milk line and black layer

From 20 to 27 daf, the seeds were immature and the grains had a liquid endosperm (milky texture). At 27 daf, there was decrease of the ML of 15%, from the crown to the pedicel of the grain. At 34 daf, the ML recorded a 20% decrease. Regarding the harvests that took place between 48 and 69 daf (134-155 das), the ML decreased towards the pedicel around 50, 45, 25, and 10% (Figure 1).

At 20, 27, and 34 daf, the seeds were immature and, consequently, no visual proof about the presence of the BL was observed. Therefore, the ANOVA did not detect any significant difference ( $p>0.05$ ) during these development stages. During the 41-69 daf period, significant statistical differences ( $p<0.05$ ) were found. In addition, at 41 daf (127 das), a faint and greyish BL was detected in 27% of the seeds. Consequently, this period was identified as the beginning of physiological maturity (BPM).

The presence of the BL increased as the samplings progressed and, finally, it became totally dark. From 41 to 48 daf, the BL of the seeds increased 59%, while from 55 and 62 daf, it increased at a lower rate. The BL percentage for these periods reached 67.5 and 78.5%, respectively. Finally, a dark BL was completely visible in 100% of the seeds at 69 daf (Figure 2). Therefore, based on the visible black color of the BL (Figure 1), the seeds reached their PM at 69 daf —*i.e.*, 155 das. In this regard, Mancera and Ramírez (2018) pointed out that the BL is the best PM indicator. They estimated that the BL percentage in the seed should be  $>75\%$ . However, they also considered that the ML is a more practical parameter. Consequently, taking into account both morphological parameters is fundamental to draw a better inference of the PM. The visual evaluation of the F6 (Yellow  $\times$  V-54A) experimental variety showed that the plant material was immature during the first two samplings (20 and 27 daf), when the endosperm had a liquid texture (100% ML) and the seeds did not have the BL. As the samplings progressed, the endosperm started to solidify and the BL



**Figure 1.** Advance of the milk line and presence of the black layer as indicators of the physiological maturity of the F6 (Yellow×V-54A) experimental maize (*Zea mays* L.) variety. The red line shows the advance of the milk line from the crown to the pedicel of the seed. The blue arrow points out the presence of the black layer in the placenta area of the seed. A) 20 daf; B) 27 daf; C) 34 daf; D) 41 daf; E) 48 daf; F) 55 daf; G) 62 daf; and H) 69 daf.

became more noticeable as the seeds reached their PM. These results match the findings of Martínez-Álvarez (2015) and Lozano-Pérez (2021).

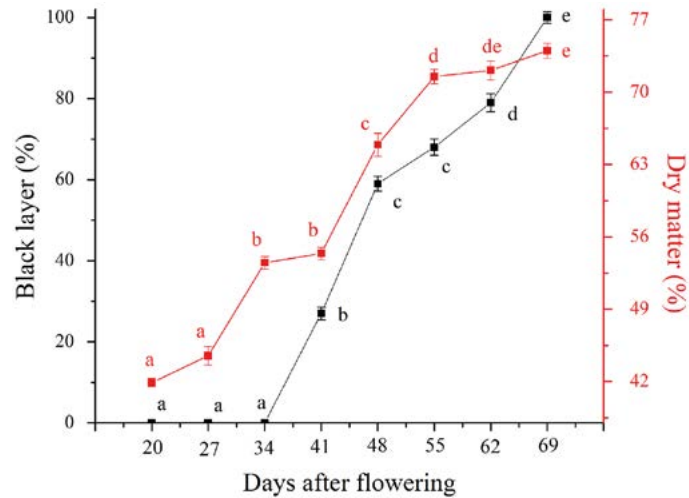
### Dry matter

The dry matter weight gain at 20 and 27 daf (41.9 and 44.5%, respectively) was not statistically significant ( $p > 0.05$ ). At 34 daf the DM increased 8.7% compared to the previous sampling, and 1.4% at 41 daf. Nevertheless, DM content did not registered statistically significant differences ( $p > 0.05$ ) between these two harvesting stages. At 48 daf, the behavior was similar than at 34 daf: DM was 10.5% higher than in the previous harvest. Finally, the grains accumulated the highest DM percentage (71.5%) at 55 daf. In addition, they continued recording a lower accumulation until they reached their physiological maturity (Figure 2).

Zavala-Hernández *et al.* (2015) reported that maximum dry matter accumulation is an indicator of the PM, while Ferreira *et al.* (2013) stated that PM is associated with the presence of the BL in all the seeds and the disappearance of the ML. In this study, the seeds reached the PM with a 74.6% dry matter accumulation and 100% BL. These results match the findings of Bewley *et al.* (2013), who linked dry matter content with the physiological maturity of the seeds.

### Moisture content

The seeds recorded 58.1% MC at 20 daf; however, this percentage decreased by 2.7% at 27 daf (Figure 3). No statistically significant difference was recorded in both cases ( $p > 0.05$ ).

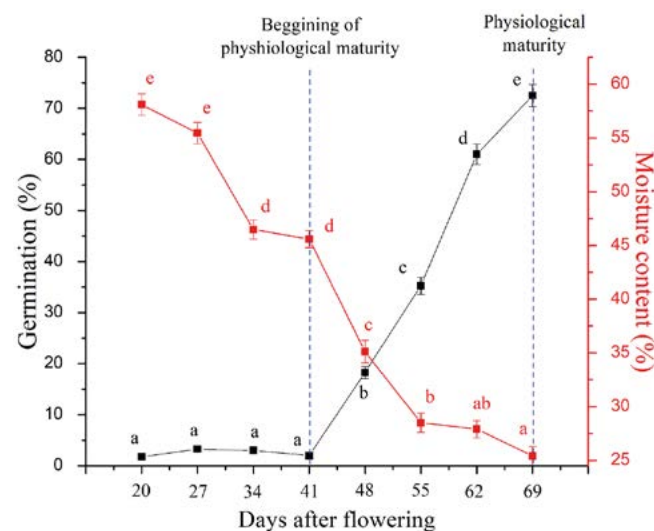


**Figure 2.** Dry weight gain and presence of the black layer (BL) during the maturation period of the seeds of the F6 (Yellow×V-54A) experimental maize (*Zea mays* L.) variety. Mean value ± standard error. Means with different letters in each sampling are statistically different (Tukey,  $\alpha=0.05$ ).

From 34 to 41 daf, MC decreased by 0.9%, recording 46.5 and 45.6%, respectively. Likewise, no statistically significant differences ( $p>0.05$ ) were recorded between them.

From 20 to 41 daf, MC decreased 12.5%. This last period was established as the BPM of the seed, given the appearance of a faint BL (27%) (Figures 1 and 2). From 48 to 69 daf, MC decreased 10.4, 17.1, 17.7, and 20.2% in relation to the BPM. Consequently, the F6 (Yellow×V-54A) experimental variety reached its PM when the seeds recorded a 25.4% MC (Figure 3), 100% BL, 10% ML, and 74.6% DM.

MC could be one of the main causes of seed deterioration: as it increases, a significant germination decrease takes place, as a result of the increase of cellular respiration (Estrada-



**Figure 3.** Relation between the germination percentage and moisture content during the maturation process of F6 (Yellow×V-54A) experimental maize (*Zea mays* L.) variety. Mean value ± standard error. Means with different letters in the eight samplings are statistically different (Tukey;  $\alpha=0.05$ ).

Urbina, 2022). Magdaleno-Hernández *et al.* (2020) pointed out that, during the formation and maturation processes, maize seeds undergo physiological, chemical, and physical changes (such as the increase of cellular respiration), which directly impact germination. For their part, Ghassemi-Golezani *et al.* (2011) mentioned that the maximum physiological quality of maize seeds is related to their maximum dry matter accumulation and their lower moisture content. Consequently, the lack of germination during the first four samplings (from 20 to 41 daf) could be the result of a high MC (58.1-45.6%), a doughy-liquid endosperm (100-50% ML), and a low BL presence (0-27%).

Likewise, this study recorded that germination reached 72.5% when MC decreased to 25.4%. These results match the reports of Mishra *et al.* (2023), who linked the physical with the physiological elements, because moisture content highly impacts seed maturity degree and quality. According to Sripathy and Groot (2023), dry matter accumulation — an indicator of physiological maturity— is the best sign of maximum quality seeds.

### Physiological quality

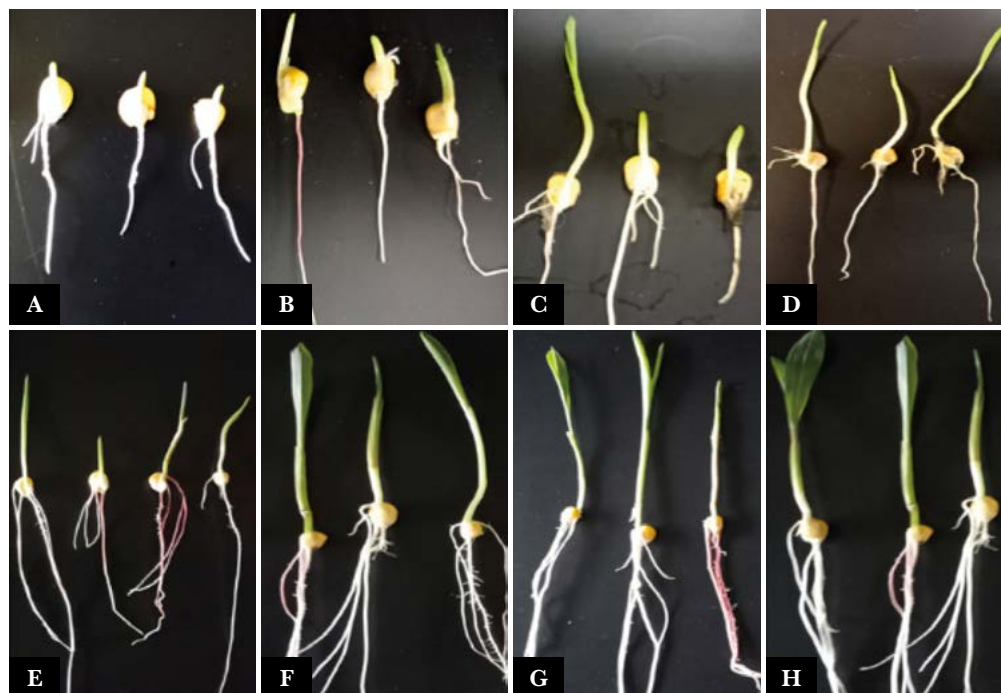
Germination was not statistically different at 20 (1.8%), 27 (3.3%), 34 (3.0%), and 41 daf (2.0%). However, it increased by 16.3% at 48 daf (Figure 3). The 41 daf harvest was established as the BPM, recording a 27% BL and a 50% decrease of the ML in the seeds. Meanwhile, germination increased 70.5% from the BPM to the PM of the seed (69 daf). Consequently, the seeds of the plant material used in this study reached their PM with a 72.5% maximum germination. In this regard, the physiological quality of maize seeds depends on their maturation degree. Therefore, in contrast with immature seeds, a higher germination is achieved when the seeds reach their PM (Escobar-Álvarez *et al.*, 2024b).

However, MC affected germination percentage of the F6 (Yellow×V-54A) experimental variety (Figure 3). This situation was the result of the maturation process of the seeds, which is highly influenced (88%) by their moisture content (Estrada-Urbina *et al.*, 2023b). Consequently, the high MC of the seeds diminished their germination. Likewise, Rosabal-Ayan *et al.* (2014) mentioned that germination involves cellular (enzymatic activation) and genetic changes (protein hydration, respiration, cell proliferation, or gene activation). Immature seeds cannot produce new plants, because they are not fully developed; meanwhile, the food material translocation to the seed stops during its physiological maturity, reaching its highest quality level. However, the moisture content is still too high (20%) to store the seeds (Dayal *et al.*, 2014).

### Normal seedlings

Figure 4 shows the growth and development evolution of the normal seedlings, from their earliest stages to their PM. Figures 4A and 4B show that the seedlings were underdeveloped when the seed was immature. In the case of the first and second samplings, the coleoptile had a <20.0 mm development, growing longer as the subsequent harvests took place. Figures 4C and 4D show a better coleoptile development. The emergence of the cotyledonary leaf is evident.

The coleoptile of some seedlings had a slight malformation (Figures 4C and 4D). This situation is the consequence of the position of the seeds in the Anchor<sup>®</sup> paper towels: adjacent

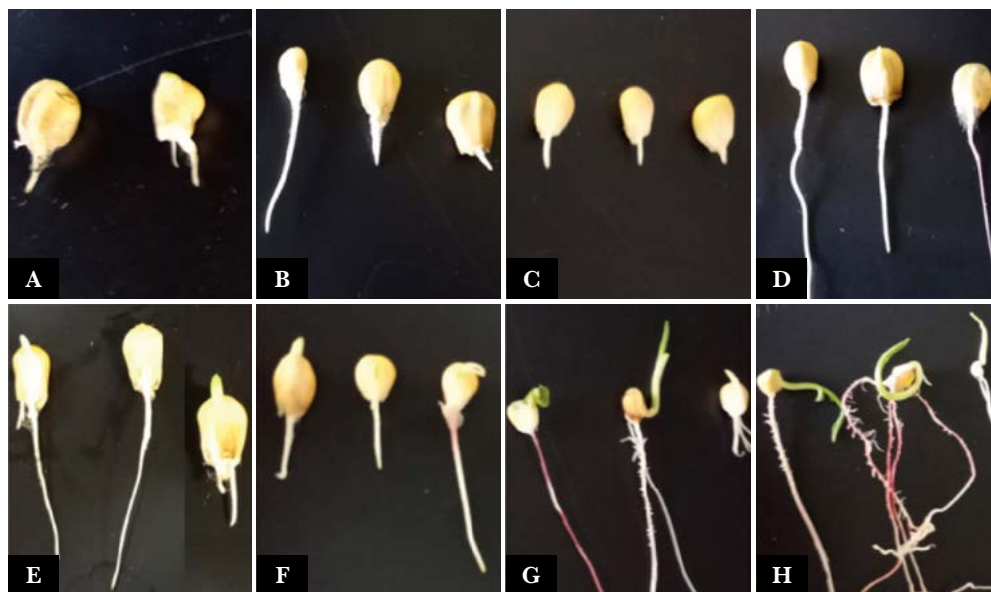


**Figure 4.** Growth and development of normal seedlings from the germination test of the F6 (Yellow×V-54A) experimental maize (*Zea mays* L.) variety. A) 20 daf; B) 27 daf; C) 34 daf; D) 41 daf; E) 48 daf; F) 55 daf; G) 62 daf; and H) 69 daf.

seeds served as a barrier for the upright growth of the seedlings and, consequently, these seedlings were not classified as abnormal (ISTA, 2021b). In addition, the secondary roots grew gradually. In the samplings carried out after the BPM (48 daf), the seedlings showed a better development of the secondary roots and primary leaves (Figures 4E and 4H). The cotyledonary leaf and the first true leaf appeared at 55 and 62 daf, respectively. Once the seeds reached their PM, the seedlings showed their maximum growth and development potential, in comparison to the previous stages when the seeds were both immature and starting to mature. Escobar-Álvarez and Estrada-Urbina (2024a) visually detected the differences regarding the quality of the seedlings from maize seeds with two degrees of maturation. The seedlings from PM seeds were more vigorous, since the development of the cotyledonary leaf and the secondary (coronary and seminal) roots were fundamental to determine their quality.

### Abnormal seedlings

Figure 5 shows that the coleoptile growth and development were inhibited from 20 to 34 daf. The Figure also includes the emergence of a 3.0-12.0 mm primary root (with a damaged root cap) and the lack of development of secondary roots (Figures 5A, 5B, and 5C). Regarding the BPM (41 daf), the main root was approximately eight times bigger than in the previous sampling. The root system was damaged and no secondary root development was recorded. The coleoptile development remained difficult until 55 daf and, in this same structure, the filling of leaf primordia never exceeded 50% (Figures 5D, 5E, and 5F).



**Figure 5.** Growth and development of abnormal seedlings from the germination test of the F6 (Yellow X V-54A) experimental maize (*Zea mays* L.) variety. A) 20 daf; B) 27 daf; C) 34 daf; D) 41 daf; E) 48 daf; F) 55 daf; G) 62 daf; and H) 69 daf.

Foliar structures grew in the coleoptile when the seeds reached a more advanced maturity (62 daf). Even during this stage and until their PM, the coleoptile of some seedlings was deformed (rolled-up shape), while other seedlings grew laterally or with a positive geotropism.

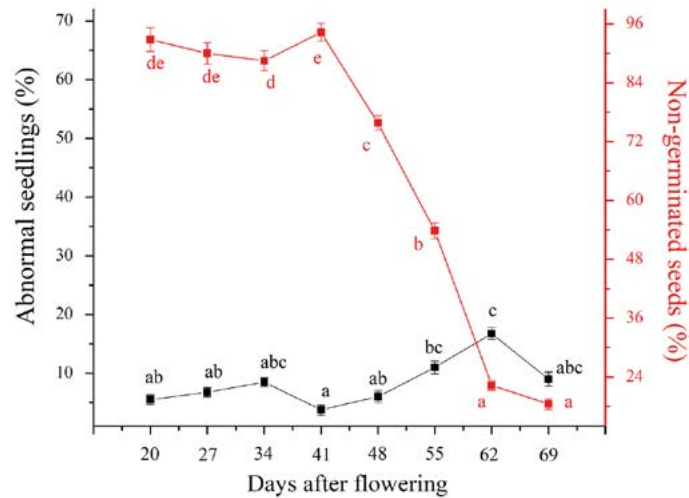
In addition, malformations and maldevelopment of the primary and secondary roots (Figures 5G and 5H) were identified. All these malformations are included in the ISTA (2021b) rules for the germination test. Clear malformations, deficiencies, and weakness in the root system and foliar structures were observed. These seedlings were classified as abnormal, because their main structures were deformed and prevented their optimal development, from the beginning of their maturity to their PM (Guillén-de la Cruz *et al.*, 2018).

During the germination test, all seedlings without main structures, malformed, badly damaged, sick, or rotten, were classified as abnormal and were not taken into account for the germination percentage. These seedlings were not expected to become normal plants, impacting their growth in the fields (Vujosevic *et al.*, 2018).

The ABNOR variation recorded statistically significant differences ( $p < 0.05$ ) throughout the evaluation period. The highest percentages of abnormalities were recorded at 55 and 62 daf: 8.5 and 16.8%, respectively (Figure 6). The abnormal seedlings recorded the same behavior pattern during the experiment. This pattern was divided into two periods: i) 20-41 daf and ii) 48-69 daf. The percentage of ABNOR was lower during the first period and it increased during the BPM.

### Non-germinated seeds

The percentage of non-germinated seeds (NOGS) fluctuated between 88.5 and 94.3% from 20 daf to the BPM. However, from 48 to 62 daf, NOGS decreased  $\approx 72\%$ . Therefore,



**Figure 6.** Morphological alteration of the maize seeds maturation of the F6 (Yellow×V-54A) experimental maize (*Zea mays* L.) variety alteration recorded in the germination test. Mean value  $\pm$  standard error. Means with different letters in the eight samplings are statistically different (Tukey,  $\alpha=0.05$ ).

NOGS at 48, 55, and 62 daf were statistically significant between each period ( $p<0.05$ ), recording 75.8, 53.8, and 22.3%, respectively. Finally, NOGS recorded 19% when the seeds reached their PM (Figure 6). In this regard, immature seeds reached the highest ABNOR and NOGS percentages in this study, as a result of the lack of the nutrients required to complete the development process. Consequently, the embryo could not reach a satisfactory growth (Alves *et al.*, 2018). Likewise, the high MC could promote a high metabolic activity (cellular respiration) that resulted in seed deterioration —*i.e.*, a low physiological quality (Pardo-Varela, 2016). One of the factors that contribute to a high physiological quality of the seeds is a timely harvest. There are several adverse environmental conditions, which accelerates the deterioration process of the seeds during their maturation; consequently, it is important to identify the right moment of the harvest of seeds. This activity (harvest) is usually associated to the physiological maturity of the seeds. However, seeds have their highest moisture content in that stage, hindering the harvesting process (Sripathy and Groot, 2023). This research suggests that the best time to the seed harvest is when they reach the physiological maturity. On the other hand, Estrada-Urbina *et al.* (2023b) indicate that maturity does not match maximum physiological quality and that the high seed quality is highly dependent on moisture content.

## CONCLUSIONS

As moisture content decreased, the seeds of the F6 (Yellow×V-54A) experimental variety recorded a higher physiological quality. The maximum quality of the product depended on the moisture content of the seed and, consequently, the germination percentage was not associated with physiological maturity. Finally, depending on seed maturity, a higher percentage of normal seedlings was recorded.

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