RESEARCH ARTICLE

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A promising method for selecting imazamox-resistant sunflower plants

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Abstract
This study was conducted to investigate the potential of using the germination test as a model for screening imazamox resistance in sunflower plants . A standard germination test was performed by applying different doses of imazamox (control, 0.1, 0.2, 0.4, 0.8, and 1.6 mM) to imidazoline-resistant (IMI-R) and susceptible (IMI-S) sunflower hybrids. Germination percentage, mean germination time, germination index, shoot length, root length, seedling fresh and dry weight, and phytotoxicity percentage for seedling growth parameters were investigated. The results showed that imazamox concentrations slightly affected the germination percentage of two sunflower hybrids at only 1.6 mM. Increasing doses of imazamox led to an increase in the mean germination time and a decrease in the germination index for both sunflower hybrids, following a similar trend. Seedling growth parameters such as shoot length, root length, and seedling fresh weight were significantly decreased by increasing imazamox doses. In addition, the differences between IMI-R and IMI-S sunflower hybrids were very evident for these parameters. The IMI-S sunflower hybrid showed sensitivity in the presence of imazamox, while no changes in the IMI-R hybrid were determined up to 0.4 mM. The inhibition percentage was higher in the IMI-S hybrid than in the IMI-R. It was concluded that the optimal dose of imazamox for the selection of resistant plants during the early growth stage was found to be 0.2 mM. The germination parameters were not good criteria for imazamox resistance, while root length, shoot length, and seedling fresh weight should be considered as selection criteria for resistance to imazamox in sunflower.
Keywords: *Helianthus annuus* L., Herbicide, Resistant, Germination, Root length

INTRODUCTION

In Türkiye, vegetable oil production depends mainly on sunflower (*Helianthus annuus* L.), which has been cultivated in arid and semi-arid regions under rainfed conditions. It has high adaptability and a high content (40-50%) quality oil in the seeds. Sunflower is affected by several adverse environmental conditions, such as drought, extreme temperature, salinity, weeds and broomrape (*Orobanche cumana* Wall.). It does not cope with the weeds due to its slow growth habits during emergence and early growth stages (Simic et al., 2011). In addition, the wide row spacing of 70 cm, which allows for lower planting densities across the field surface, makes it more susceptible to weed infestation. To solve weed management, IMI-resistant (IMI- R) sunflower hybrids have been developed, which allow the use of imidazolinone herbicides to control a wide variety of weeds in sunflower (Kaya and Kolsarıcı, 2005; Stefanic et al., 2023).

Imazamox, an imidazolinone (IMI) herbicide, is used post-emergent in leguminous crops and IMI-resistant wheat, sunflower, rice, lentil, and canola cultivars (Breccia et al., 2020). This herbicide effecctively controls both narrow and broadleaf weeds. The broomrape, a parasitic weed in plants belonging to *Asteraceae* species, is known as a sunflower parasite, which is one of the major factors destroying sunflower production (Mitkov et al., 2019). However, imazamox applications are highly effective in controlling broomrape along with other weed species (Mitkov et al., 2019; Shaner et al., 2019). For these reasons, the primary broadleaf, grass weed, and parasitic weeds from the genus *Orobanche* can be effectively controlled by producing hybrids of sunflowers that are resistant to imazamox (Fernandez-Martinez et al., 2009; Malidza et al., 2003).

Routinely, the seeds are sown and sprayed with imazamox herbicide at an appropriate dose when the plants are at V4-V8 stages in order to identify imazamox-resistant sunflower plants. Visual inspection is performed at 7 and 14 days after application (at score 0, there are no damages on the crop, and at score 9, the crop is completely destroyed) (Neshev et al., 2020). However, this method is an expensive, labor-intensive, and time-consuming process for early selection of IMI resistance. A pre-selection method should be developed to screen for resistant plants before field trials. For this reason, this study was undertaken to establish a simple, rapid, and effective model for the separation of imazamox susceptible and resistant sunflower plants using germination and early seedling growth under laboratory conditions.

MATERIALS AND METHODS

A standard germination test was performed to identify imazamox-resistant sunflower plants by comparing the germination and early seedling growth performance of resistant and susceptible sunflower hybrids at the Seed Science and Technology Laboratory, Eskişehir Osmangazi University, in 2024. Two commercial sunflower hybrids, the imidazolinone-resistant (IMI-R) hybrid SY Roseta CLP and the susceptible (IMI-S) hybrid SY Gibraltar, were used in this study.

The seeds were germinated at five levels of 0.1, 0.2, 0.4, 0.8, and 1.6 mM imazamox, prepared from Intervix Pro® with 40 g imazamox per liter. Distilled water was used for control. Four replicated fifty seeds from each sunflower hybrid were placed between three filter paper sheets with a dimension of 20 × 20 cm and each paper was watered with 7 mL of the respective imazamox solutions. After rolling filter papers, they were put into sealed plastic bags and transferred to an incubator at 25 °C for 8 days under dark conditions. Two millimeters of radicle protrusion was considered the germination criterion (ISTA 2018). Germination percentage (GP), mean germination time (MGT), and germination index (GI) were calculated as Equations 1, 2, 3, and 4:

$$GP\ (\%) = \frac{Germinated\ seeds\ at\ final\ day}{Total\ seeds}\ \text{x 100, (ISTA, 2018) (1)}$$

$$MGT\ (day) = \frac{\sum Dn}{\sum n}\ \text{(ISTA, 2018) (2)}$$

where, n is the seed number germinated on day D, and D is the number of days from the beginning of the germination test.

$$GI = \frac{Number\ of\ germinated\ seeds}{Days\ of\ the\ first\ count} + \cdots + \frac{Number\ of\ germinated\ seeds}{Days\ of\ the\ final\ count}\ \text{Salehzade et al. (2009) (3)}$$

On the last day (8th day), ten seedlings randomly selected from each imazamox level were sampled to determine shoot length (SL), root length (RL), and seedling fresh (SFW) and dry weights (SDW) after they were dried in an air oven at 80 °C for 24 h. The inhibition percentage was determined using the formula described by Archana et al. (2016).

$$\%\ inhibition = \frac{Control - Test\ solution}{Control}\ \text{x 100 (4)}$$

Statistical Analysis

The experiment was set up in a two-factor factorial in a completely randomized design with four replicates. Data were analyzed using the computer program MSTAT-C (v. 2.10, Michigan State University), and the differences were compared using the Least Significant Differences (LSD) test at the 5% level. A quadratic regression equation (was used to calculate the toxic level (50% reduction from control) of imazamox for each parameter (Harrison et al., 1976). In the regression equation, the dependent variable, y, was set to 50% of the related parameters in the control group.

RESULTS

A significant interaction between sunflower hybrids and imazamox doses was found for all parameters except germination percentage (Table 1). Germination percentages of the sunflower hybrids were similar, but increased imazamox doses significantly decreased at 1.6 mM. The mean germination time of the IMI-R and IMI-S hybrids was different and prolonged with increasing doses of imazamox. A similar trend was observed for two hybrids, with IMI-R germinating faster than IMI-S (Figure 1A). Also, a higher germination index was calculated in IMI-R at all levels of imazamox, but it was reduced by increasing imazamox (Figure 1B). There was a significant difference in shoot length between IMI-S and IMI-R sunflower hybrids at all doses of imazamox. Shoot length was sensitive to imazamox treatments because differences between sunflower hybrids were evident. The shoot length of IMI-S was greatly reduced in the presence of imazamox, however, it was significantly decreased in the IMI-R hybrid at 0.4 mM (Figure 1C). The toxic levels of imazamox were calculated as 1.04 mM and 0.57 mM for IMI-R and IMI-S hybrids, respectively, using the regression equations in Figure 1C. Imazamox caused a reduction in root length of sunflower hybrids. In the presence of imazamox, the root length of the IMI-S hybrid was apparently decreased, but a significant reduction in IMI-R hybrid was observed at 0.4 mM. In both sunflower hybrids, the minimum root length was obtained at the imazamox dose of 1.6 mM, which was similar to each other (Figure 1D). A 50% reduction in root length was estimated to be 0.92 mM for IMI-R and 0.24 mM for IMI-S hybrid. The seedling fresh weight varied depending on the reduction in root and shoot length,. IMI-R sunflower hybrid produced a higher seedling fresh weight than IMI-S, while it was adversely affected at higher levels than 0.4 mM (Figure 1E). The toxic concentrations of imazamox for the fresh weight of the seedlings of the IMI-R and IMI-S hybrids were 0.52 mM and 0.55 mM, respectively. There was a significant difference between sunflower hybrids regarding seedling dry weight in control. However, imazamox increased the dry weight of the IMI-R sunflower hybrid and exhibited heavier dry weight under high doses of imazamox.

As expected, inhibition percentages for shoot length, root length, and seedling fresh weight of IMI-S were higher than those of the IMI-R hybrid (Figures 2A, B and C). At 0.1 mM imazamox, the IMI-S hybrid had the highest inhibition percentages. However, the inhibition percentage of the IMI-R hybrid was significantly increased at 0.4 mM, and higher concentrations of imazamox induced the inhibition percentage.

Table 1. Analysis of variance and main effects of imazamox doses on the investigated characteristics of IMI-S and IMI-R sunflower hybrids.

Factors	GP (%)	MGT (day)	GI	SL (cm)	RL (cm)	SFW (mg plant^{-1})	SDW (mg plant^{-1})
Hybrids (A)							
IMI-S	86.6	2.20^{a}	20.8^{b}	3.31^{b}	2.15^{b}	2585^{b}	519^{a}†
IMI-R	86.5	1.97^{b}	24.3^{a}	6.55^{a}	7.77^{a}	4086^{a}	480^{b}
Imazamox doses (B)							
Control	85.7^{b}	2.01^{c}	22.5^{b}	6.80^{a}	8.15^{a}	4432^{a}	499^{b}
0.1 mM	91.0^{a}	2.01^{c}	25.0^{a}	5.36^{c}	6.51^{b}	3503^{c}	489^{b}
0.2 mM	88.5^{ab}	1.99^{c}	24.9^{a}	5.98^{b}	6.65^{b}	3702^{b}	503^{ab}
0.4 mM	87.5^{ab}	2.13^{b}	22.2^{b}	4.38^{d}	3.88^{c}	3188^{d}	494^{b}
0.8 mM	85.5^{b}	2.16^{ab}	21.1^{c}	4.15^{d}	2.84^{d}	2742^{e}	498^{b}
1.6 mM	81.2^{c}	2.23^{a}	19.6^{d}	2.89^{e}	1.73^{e}	2449^{f}	516^{a}
Analysis of variance							
A	*ns*	**	**	**	**	**	**
B	**	**	**	**	**	**	*
A×B	*ns*	*	**	**	**	**	**

significant at 1%, *ns*= non-significant, †= Letter(s) connected with the means denote significance levels at P<0.05.

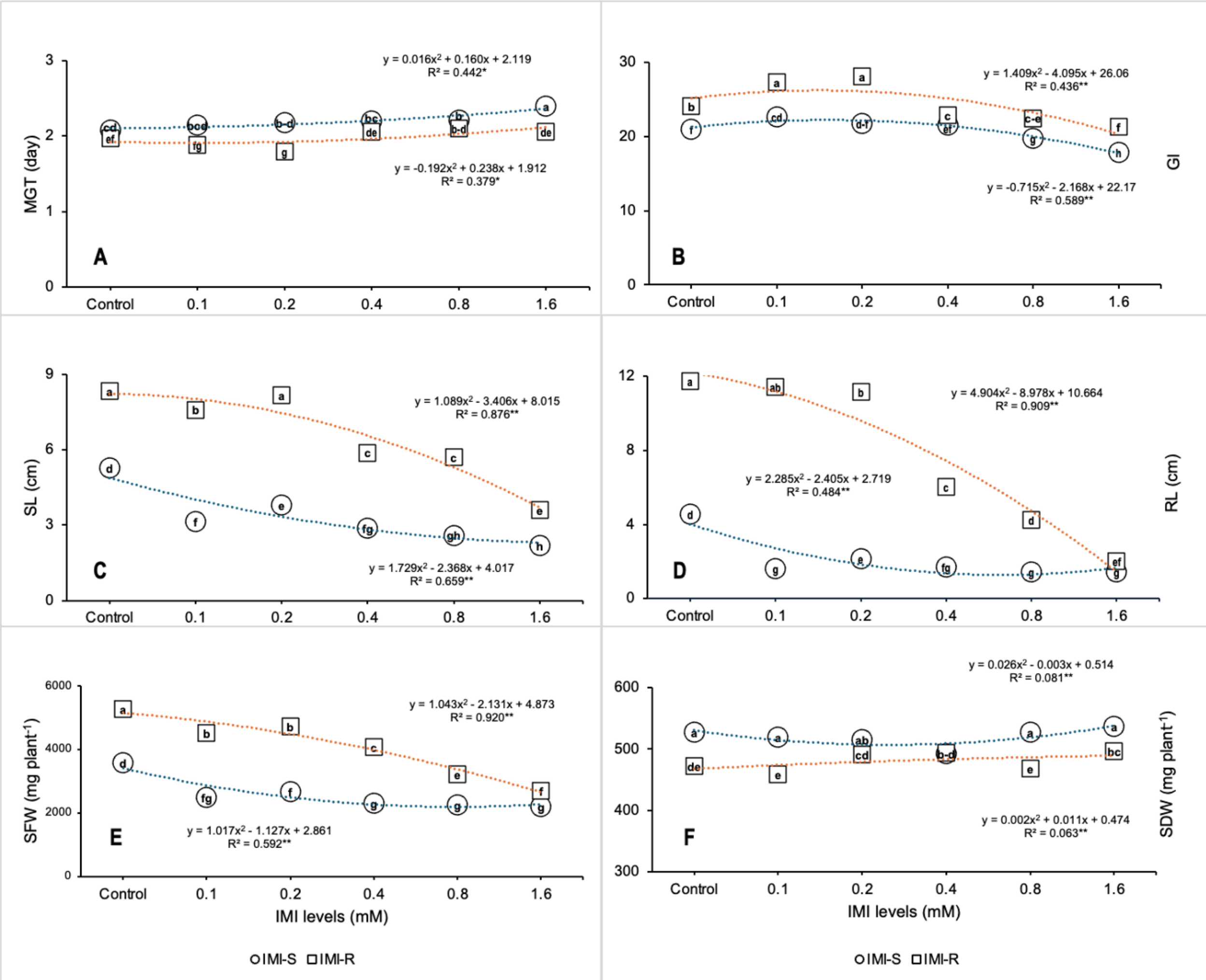

Figure 1. Changes in the investigated parameters of IMI-S and IMI-R sunflower hybrids exposed to increasing imazamox doses.

Table 2. Inhibition percentage of shoot length, root length, and seedling fresh weight of IMI-S and IMI-R sunflower hybrids

Factors	**Inhibition %**		
	SL	**RL**	**SFW**
Hybrids (A)			
IMI-S	37.1^{a}	52.7^{a}	27.9^{a}
IMI-R	21.4^{b}	33.8^{b}	22.5^{b}
Imazamox doses (B)			
Control	-^{f}	-^{f}	-^{f}
0.1 mM	24.6^{d}	33.8^{d}	22.5^{d}
0.2 mM	14.9^{e}	28.9^{e}	17.9^{e}
0.4 mM	37.3^{c}	55.4^{c}	29.2^{c}
0.8 mM	41.1^{b}	66.0^{b}	37.9^{b}
1.6 mM	57.6^{a}	75.5^{a}	43.7^{a}
Analysis of variance			
A	**	**	**
B	**	**	**
A×B	**	**	**

significant at 1%, *ns*= non-significant, †= Letter(s) connected with the means denote significance levels at P<0.05.

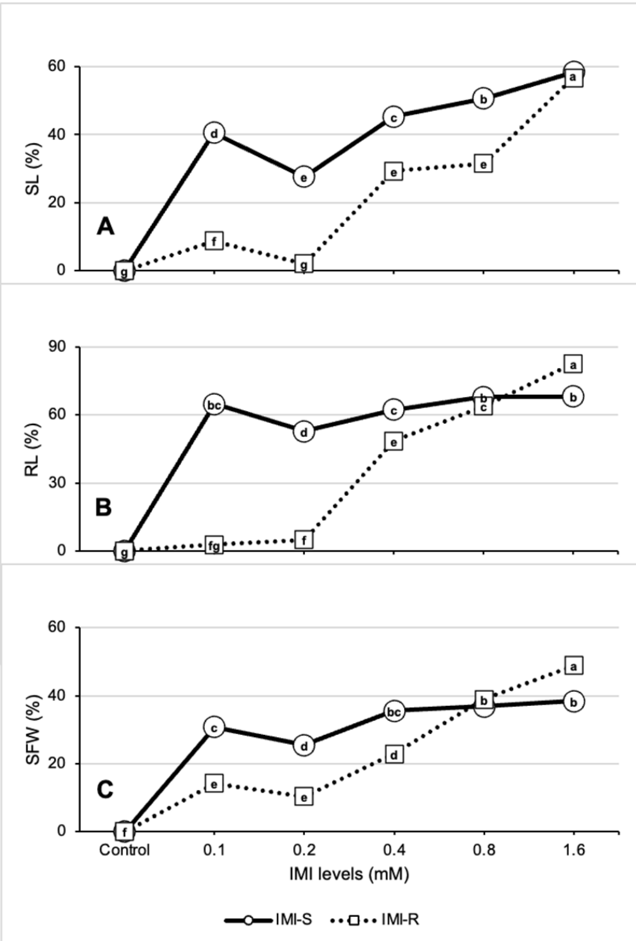

Figure 2. Inhibition percentages of shoot length, root length and seedling fresh weight of IMI-R and IMI-S sunflower hybrids under different imzamox concentrations.

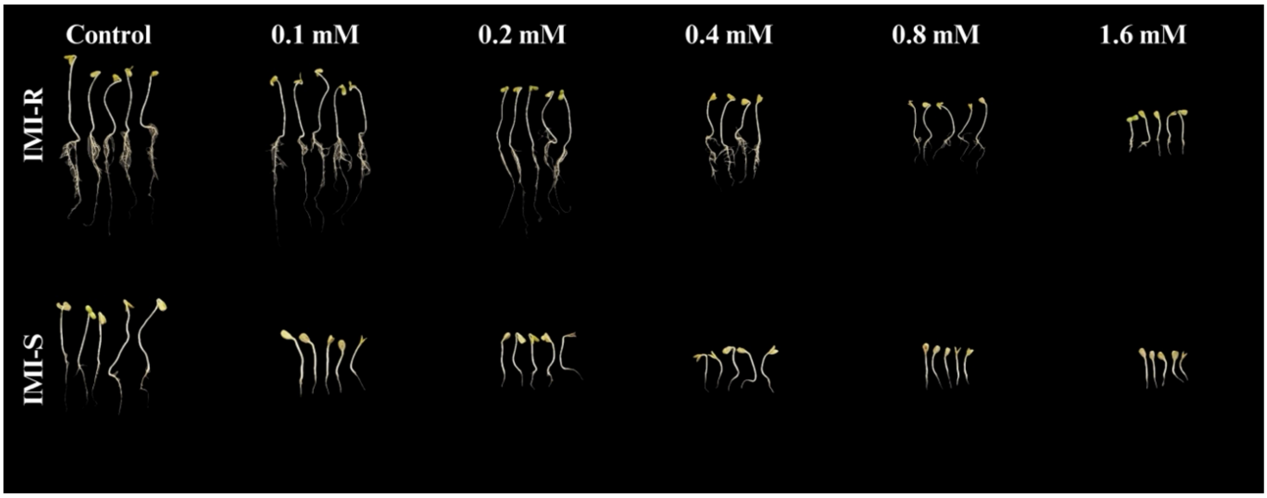

Figure 3. Changes in seedling growth of IMI-R and IMI-S sunflower hybrids subjected to different doses of imazamox.

DISCUSSION

This study showed that the germination percentage of IMI-S and IMI-R sunflower hybrids was not affected by low and medium doses of imazamox but was slightly inhibited by an overdose (1.6 mM). However, the mean germination time was delayed and the germination index was reduced as the imazamox concentration was increased. These parameters showed similar behavior in IMI-S and IMI-R sunflower hybrids, but there was no significant separation between resistant and susceptible hybrids. Our results confirmed the findings of Haliloğlu et al., (2022) in wheat, who found that germination percentage was not changed by imazamox doses under different germination mediums. These results suggest that imazamox toxicity begins after germinating seeds absorb water through the roots.

Seedling growth characteristics, including shoot length, root length, and seedling fresh weight, were severely inhibited in the presence of imazamox in the IMI-S sunflower hybrid. In contrast, the IMI-R hybrid exhibited no observable appearance up to a dose of 0.4 mM imazamox. The sensitivity of root growth in sunflower IMI-S was pronounced, with depletion observed at 0.1 mM imazamox. A clear difference was determined between the root lengths of the IMI-S and IMI-R hybrids (Figures 1D and 2B). This result is consistent with the findings of Breccia et al., (2018), who found that imazamox-sensitive wheat cultivars showed a sharp decrease in root length in the presence of imazamox. Similarly, shoot length responded to imazamox and successfully separated the IMI-R from the IMI-S hybrid. The availability of imazamox resulted in decreased shoot length in the IMI-S hybrid, while it was reduced at 0.4 mM in the IMI-R hybrid. Imazamox reduced the fresh weight ofseedling , but the response of sunflower hybrids varied basedthe length of shoot and root. Similar findings were observed in wheat by Breccia et al., (2018) and Haliloğlu et al., (2022), who found that root and shoot growth were inhibited by increasing doses of imazamox. Coversely, this study did not report any dead seedlings. Therefore, the duration of the experiment was terminated at 8 days because the sunflower seedlings in the control started to deteriorate, which was not enough time for the seedlings to die.

The inhibition percentage for root length, shoot length, and seedling fresh weight was higher in the IMI-S than in the IMI-R hybrid, and they provided a more accurate selection of imazamox-resistant plants from susceptible ones (Figure 2). Surprisingly, the inhibition percentages of IMI-R increased continuously as imazamox concentrations increased.

CONCLUSION

These results indicated that germination parameters could not be used as selection criteria for sunflower plants resistant to imazamox. Seedling growth was much more sensitive to imazamox, and the IMI-S hybrid was more affected by imazamox than the IMI-R hybrid. Root length, shoot length, and seedling fresh weight should be successfully evaluated to identify imazamox-resistant genotypes in sunflower germplasm. In addition, the percentage of phytotoxicity for seedling growth characteristics could be used as valuable criteria for imazamox resistance. Imazamox concentrations of 0.2-0.4 mM should be preferred for screening the sunflower genotypes.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declared that there is no conflict

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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Data availability

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

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