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RESPONSE OF JATROPHA CURCAS SUPERIOR CLONES TO DROUGHT STRESS

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ABSTRACT

Superior clones of *Jatropha curcas* that will be released as superior varieties must be tested for drought stress tolerance. This study aims to examine the drought stress tolerance of superior clones of Jatropha. The study took place in the Green House of the Indonesian Sweetener and Fiber Crops Research Institute in Malang, East Java, Indonesia, from January to December 2021. The treatments were arranged in a split-plot design with 3 replications. The main plot consisted of 4 soil moisture levels (40, 60, 80, and 100% of field capacity), and the subplots consisted of 4 superior clones (HS-49xSP-65/32, IP-3AxSP-89/4, IP-3AxSP-65/11, IP-3PxSP-7/5) and a control clone (Jet-1 Agribun). The results showed that the clones tested were classified as susceptible – tolerant to water stress. Clones HS-49xSP-65/32 are classified as tolerant, clones IP-3AxSP-65/11 and IP-3PxSP-7/5 are classified as moderate, and clones IP-3AxSP-89/4 and Jet-1 Agribun are classified as susceptible.

KEY WORDS

Jatropha, tolerance, susceptible, stress, drought.

Jatropha (*Jatropha curcas* L.) is a multi-purpose shrub native to tropical America (Pecina-Quintero et al., 2014). These plants spread to tropical and subtropical areas in Africa and Asia (Mardhiah et al., 2017) and grow on marginal lands (Contran et al., 2013) with low rainfall and hot climates (Alburquerque et al., 2017).

Jatropha growth varies greatly; seed yields range from 0.2-2.0 kg per plant (Corte-Real et al., 2016). In 2005, Indonesia developed Jatropha as a cultivated plant on dry land with an annual seed productivity of approximately 115 kg/ha (Syakir, 2010). This number is lower than the yield potential of one-year-old Jatropha seeds on dry land of 880 kg/ha (Santoso et al., 2008). On the other hand, Jatropha farming is considered good if seed productivity reaches 7 tons/ha (Syakir, 2010). Therefore, it is necessary to make efforts to increase the productivity of jatropha in a dry land.

The limiting factors for Jatropha cultivation in dry land are limited water supply and low soil fertility. Such conditions hinder the growth of Jatropha (Gedoan et al., 2011). On the other hand, available planting materials such as IP-1, IP-2, and IP-3 were produced from the selection of Jatropha under sufficient water conditions. The incompatibility of planting materials with dry land conditions causes low crop productivity. There are still a few new varieties of Jatropha as superior planting material. Therefore, it is necessary to find suitable planting materials for dry land conditions to obtain high crop productivity from existing superior varieties.

Genetic improvement of Jatropha has been carried out through either provenance selection, crosses, gene mutations, or transgenics. The selection of superior provenances in 2007-2009 on dry land resulted in three clones with high productivity, namely HS-49/NTT, PT-7/Lampung, and NTB-3189 (Sudarmo et al., 2010). One of these clones has been released as a new superior variety named Jet-1 Agribun. In 2010, crosses between genotypes were carried out, and the results showed 6 combinations of crosses had very high positive heterosis and heterobeltiosis values, namely HS-49xSP-65, HS-49xSP-54, HS-49xSP-103, IP-3PxSP-7, IP -3AxSP-65, and IP-3AxSP-89 (Purwati et al., 2020). Follow-up selection was conducted in 2014, and 15 selected hybrid clones were obtained. In 2015-2017, yield tests were done, and 9 superior hybrid clones with high productivity, including HS-49xSP-65/32, IP-3AxSP-89/4, and IP-3AxSP-65/11, were produced (Purwati et al., 2018). Then the selected clones were tested in multilocation as a requirement for releasing varieties. In releasing varieties, supporting data is needed, such as plant response to drought stress. Therefore, it is necessary to test the tolerance of superior clones to water stress to know the tolerance level in each clone. A tolerance test aims to determine the response of each superior Jatropha clone to drought stress.

MATERIALS AND METHODS OF RESEARCH

The study took place in the Green House of the Indonesian Sweetener and Fiber Crops Research Institute in Malang, East Java, Indonesia, from January to December 2021. The planting material was stem cuttings from 5 jatropha clones. The stem cuttings had a diameter of ±2 cm and a length of 30 cm and were planted in polybags. The polybag used contained 20 kg of wind-drying soil with medium-textured Inceptisols soil. Plants grown and were about 4 months old after planting were treated according to the treatment. The tools included measuring cups, gypsum meters, gypsum blocks, scales, an oven, and a ruler.

Treatments and cultivation methods. Treatments were arranged in a split-plot design with three replications. The main plot consisted of four soil moisture levels (40, 60, 80, and 100% of field capacity), and the subplots consisted of five Jatropha clones (HS-49xSP-65/32, IP-3AxSP-89/4, IP-3AxSP- 65/11, IP-3PxSP-7/5, and Jet-1 Agribun as controls). The total number of combination treatments in one replication was 20 treatments. Each treatment in one replication consisted of two plants arranged at a distance of 80 cm x 40 cm.

Before treatment, all were conditioned at 100% soil moisture (field capacity) and weighed to determine the wet weight of polybag soil (FWP). The polybag soil was allowed to dry until the humidity reached 80% unless the 100% soil moisture treatment was maintained. Furthermore, the soil was allowed to dry until the humidity reached 60%, except for the 80 and 100% treatments, which were maintained according to the treatment. Moisture reduction was repeated until it reached 40%, except for treatments 60, 80, and 100%, which were maintained according to treatment. The soil moisture stress treatment started when the soil moisture reached 40%.

Soil moisture stress treatment was carried out for two months by maintaining soil moisture according to the treatment. To maintain soil moisture, each polybag was added water. The volume of water added was adjusted according to the decrease in the humidity of each polybag. The humidity of each polybag was measured using a gypsum meter by placing a gypsum block in each polybag at a soil depth of 20 cm. Soil moisture measurements were carried out every two days. To determine the amount of water added so that soil moisture increased by 1%, soil samples were taken from each polybag at 100% soil moisture conditions. Soil samples were weighed for the wet weight (FWS) and in the oven at 80°C for 72 hours to determine the dry weight (DWS).

The amount of water in a 100% moist soil sample (TWS) is calculated by the formula:

$$TWS = FWS - DWS \text{ (ml)}$$

The amount of water contained in each polybag (TWP) is calculated by the formula:

$$TWP = \frac{FWP}{FWS} \times TWS \text{ (ml)}$$

To increase soil moisture by 1%, additional water (AW) is needed as much as:

$$AW = \frac{TWP}{100} \text{ (ml/polybag)}$$

Observation of growth variables. Observations were made after two-month treatment with the observed variables, including leaf abscission, the total number of leaves formed, and stem volume. Leaf abscission was calculated from the accumulated number of fallen leaves every day. The total number of leaves formed was calculated by accumulating the number of leaves formed every 15 days. The increase in stem volume was calculated from the difference in stem volume two months after treatment with at the beginning of treatment. Stem volume was measured from the length and diameter of each stem segment in one plant.

Plant ability to recover after experiencing water stress was measured after soil moisture in all treatments was restored to field capacity for a month. The observed variables measured included the total number of leaves formed, the increase in stem volume, and the amount of fruit formed.

Analysis of variance and Duncan's multiple range tests at a 5% level were performed to determine the effect of treatment on the variables measured.

The tolerance level of Jatropha clones was determined by calculating the Stress Sensitivity Index (SSI) for each observed variable, according to Fisher and Maurer (1978), which was modified:

$$SSI = \frac{1-(stressed\ clones/normal\ clones)}{1-(average\ stressed\ clones/normal\ clones)}$$

SSI only describes a clone as more tolerant than other clones. Modification of ranking categories needs to be done so the SSI of each clone can be grouped. Clones with the highest SSI for leaf abscission, the total number of leaves formed, increase in stem volume, and amount of fruit formed during recovery are categorized as very susceptible, while those with the lowest SSI are categorized as very tolerant. The opposite happens to the total number of leaves formed and increased stem volume during recovery. SSI between the observed variables is diverse, so a Category Threshold Value (CTV) must be determined. Five categories of plant tolerance to soil moisture stress are used so that the CTV value for each variable is calculated from the maximum SSI value (SSImax) divided by five. The clones are categorized into five based on leaf abscission, the total number of leaves formed, the increase in stem volume during stress, the amount of fruit formed during stress, the increase in stem volume during recovery, and the amount of fruit formed during recovery.

- Very susceptible if SSI ≥ (SSImax – CTV) and the index value is 1;
- Susceptible if (SSImax – 2 CTV) < SSI < (SSImax – CTV) and the index value is 2;
- Moderate if (SSI max – 3 CTV) < SSI < (SSImax – 2 CTV) and the index value is 3;
- Tolerant if (SSImax – 4 CTV) < SSI < SSImax – 3 CTV) and the index value is 4
- Very tolerant if SSI ≤ (SSImax – 4 CTV) and the index value is 5.

The average index of the six observed variables will be used to determine the tolerance level of the tested Jatropha clones.

RESULTS AND DISCUSSION

Growth and Production Components. The number of fallen leaves (abscission), the total number of leaves formed, and the increase in stem volume of Jatropha within two months was influenced by the interaction between the clone and soil moisture (Table 1, 2, and 3). Generally, all clones of Jatropha responded to a decrease in soil moisture by increasing the number of fallen leaves, decreasing the formation of new leaves, and decreasing the increase in stem volume. Water stress on Jatropha causes an increase in the synthesis of abscisic acid (ABA) in the root and an increase in the synthesis of ABA and ethylene in the leaves (Sapeta *et al.*, 2016). Increased synthesis of ABA and ethylene in the leaves lead to an increase in the number of fallen leaves and a decrease in the formation of new leaves. An increase in the number of fallen leaves, a decrease in the number of leaves formed, and a decrease in the rate of photosynthesis cause a decrease in available carbohydrates for stem growth. The same finding was shown by Fini *et al.* (2013) and Sapeta *et al.* (2013).

Under moist soil conditions at field capacity (100%), clone IP-3AxSP-65/11 had the fewest number of fallen leaves, and clone IP-3PxSP-7/5 had the highest number of fallen leaves (Table 1). However, when the soil moisture decreased to 40% of field capacity, clone IP-3PxSP-7/5 experienced the highest number of fallen leaves, and clone HS-49xSP-65/32 had the fewest number of fallen leaves. In general, clones that suffer a few leaf losses under water stress conditions are more tolerant than clones that experience a lot of leaf losses (Anjum *et al.*, 2011; Silva *et al.*, 2012). Thus, it is suspected that clone HS-49xSP-65/32 is more tolerant than the other clones, and clone IP-3PxSP-7/5 is more susceptible than the other clones tested.

Table 1 – The number of fallen leaves (in pieces) of some Jatropha clones during water stress

Soil moisture (%)	HS-49xSP-65/32	IP-3AxSP-89/4	IP-3AxSP-65/11	IP-3PxSP-7/5	Jet-1 Agribun
40	18.68 e-h	23.15 a-c	19.62 d-g	25.38 a	22.82 a-d
60	18.56 e-h	21.59 b-e	18.73 e-h	24.87 a	20.99 b-f
80	18.14 e-h	21.54 b-e	16.94 gh	24.16 ab	20.37 c-g
100	17.73 f-h	18.73 e-h	15.73 h	20.07 c-g	17.86 f-h

Note: Numbers with the same letters are not significantly different in the 5% level DMRT test.

In conditions where soil moisture was not a limiting factor for plant growth (field capacity), clones HS-49xSP-65/32, IP-3AxSP-65/11, and IP-3PxSP-7/5 were able to form the highest number of new leaves (Table 2). The decrease in soil moisture to 40% of field capacity for two months caused the IP-3PxSP-7/5 and Jet 1 clones to form the fewest new leaves, while the HS-49xSP-65/32 clone was able to form the highest number of new leaves. In general, clones that produce a lot of new leaves are more tolerant than clones that produce a few new leaves (Anjos *et al*., 2017; Maftuchah *et al.,* 2019). Thus, it is suspected that the HS-49xSP-65/32 clone is more tolerant, and the IP-3PxSP-7/5 and Jet-1 Agribun clones are more susceptible to water stress than the other clones.

Table 2 – Number of leaves formed (in pieces) of some Jatropha clones during water stress

Soil moisture (%)	HS-49xSP-65/32	IP-3AxSP-89/4	IP-3AxSP-65/11	IP-3PxSP-7/5	Jet-1 Agribun
40	6.60 f-h	4.97 gh	5.89 gh	3.80 h	3.84 h
60	12.73 e	9.45 f	12.45 e	7.20 fg	6.18 gh
80	14.61 de	16.84 cd	16.22 cd	14.13 de	15.97 cd
100	21.11 ab	18.76 bc	21.96 a	20.13 ab	18.34 bc

Note: Numbers with the same letters are not significantly different in the 5% level DMRT test.

The IP-3AxSP-89/4 and HS-49xSP-65/32 clones produced the highest increase in stem volume per plant, and the IP-3PxSP-7/5 clone produced the lowest increase in stem volume under field capacity (100%) for two months (Table 3). The decrease in soil moisture to 40% of field capacity resulted in only clone IP-3AxSP-65/11 producing the highest increase in stem volume, and clone IP-3PxSP-7/5 produced the least increase in stem volume. In general, clones that produce a large increase in stem volume during water stress are more tolerant than clones that produce a small increase in stem volume (Yin *et al.*, 2016; Abrar *et al*., 2020). Therefore, it is suspected that clone IP-3AxSP-65/11 is more tolerant to water stress than the other clones tested, while clone IP-3PxSP-7/5 is more susceptible than the other clones.

Table 3 – Increase in stem volume (cm^3) of some Jatropha clones during water stress

Soil moisture (%)	HS-49xSP-65/32	IP-3AxSP-89/4	IP-3AxSP-65/11	IP-3PxSP-7/5	Jet-1 Agribun
40	3.14 g-i	1.44 ij	3.45 f-i	0.91 j	2.36 h-j
60	4.81 d-g	4.26 e-h	5.14 d-g	2.63 h-j	3.04 g-i
80	6.82 d	7.01 d	6.53 d	5.3 d-f	6.98 d
100	11.58 ab	12.37 a	10.1 bc	5.71 de	9.24 c

Note: Numbers with the same letters are not significantly different in the 5% level DMRT test.

After two-month water stress, the moisture level was restored to field capacity. During the recovery period, all clones that had experienced 40% water stress produced the least number of fallen leaves. The clone that had experienced 60% water stress and produced the least number of fallen leaves was Jet-1 Agribun, while the highest was obtained by IP-

3PxSP-7/5 (Table 4). In general, clones that produce a small number of fallen leaves during the recovery process after experiencing water stress are more tolerant than those that produce many fallen leaves (Arcoverde *et al*., 2011; Makholwa *et al*., 2021). Thus, it is suspected that the Jet-1 Agribun clone is more tolerant than the other clones tested, while the IP-3PxSP-7/5 clone is more susceptible than the other clones.

Table 4 – The number of fallen leaves (in pieces) of some Jatropha clones during the recovery phase after experiencing water stress

Soil moisture (%)	HS-49xSP-65/32	IP-3AxSP-89/4	IP-3AxSP-65/11	IP-3PxSP-7/5	Jet-1 Agribun
40	7.01 g	5.32 g	6.95 g	7.63 g	5.98 g
60	17.01 d-e	12.69 f	14.05 ef	18.04 c-e	7.17 g
80	21.71 a-c	14.44 ef	15.27 ef	20.59 b-d	21.24 bc
100	23.10 ab	21.43 bc	25.59 a	22.92 ab	21.71 a-c

Note: Numbers with the same letters are not significantly different in the 5% level DMRT test.

During recovery, for plants that experienced no water stress (100% of field capacity), clone IP-3AxSP-65/11 was able to form the highest number of new leaves, while Jet-1 Agribun formed the fewest new leaves (Table 5). For plants experiencing water stress up to 40% of field capacity, all clones tested (except IP-3AxSP-65/11) produced the highest number of new leaves. In general, after experiencing water stress, clones that produce many new leaves during the recovery process are more tolerant than those that produce few new leaves (Duarte *et al*., 2015; Yadav *et al*., 2019). Thus, it is suspected that the HS-49xSP-65/32, IP-3AxSP-65/11, IP-3PxSP-7/5, and Jet-1 Agribun clones are more tolerant than the other clones tested, while the IP-3AxSP-65/ 11 is more susceptible than other clones.

Table 5 – Number of new leaves (in pieces) of some Jatropha clones during the recovery phase after experiencing water stress

Soil moisture (%)	HS-49xSP-65/32	IP-3AxSP-89/4	IP-3AxSP-65/11	IP-3PxSP-7/5	Jet-1 Agribun
40	27.85 ab	28.63 ab	26.39 b-d	28.15 ab	32.83 a
60	21.39 c-e	16.8 e-g	20.69 d-f	27.16 a-c	21.78 c-e
80	16.81 e-g	14.83 f-h	18.80 ef	21.54 c-e	20.17 ef
100	16.10 e-g	11.2 gh	18.79 ef	16.41 e-g	9.84 h

Note: Numbers with the same letters are not significantly different in the 5% level DMRT test.

During the recovery phase, clone HS-49xSP-65/32, previously grown on moist soil at field capacity, produced the highest increase in stem volume per plant, while clone IP-3PxSP-7/5 produced the least (Table 6). However, during the recovery phase, clones HS-49xSP-65/32, IP-3AxSP-65/11, and Jet-1 Agribun, previously treated under soil moisture of 40% of field capacity, produced the highest increase in stem volume per plant, while IP-3PxSP-7/5 produced the least. In general, clones grown under water-limited conditions produce a large increase in stem volume during the recovery phase and are more tolerant to water shortages than clones that produce a small increase in stem volume (Carneiro *et al*., 2015; Contran *et al*., 2017). Therefore, HS-49xSP-65/32, IP-3AxSP-65/11, and Jet-1 Agribun clones are suspected to be more tolerant than the other clones tested, while IP-3PxSP-7/5 clone is more susceptible than the other clones.

Table 6 – Increase in stem volume (cm^3) of some Jatropha clones during the recovery phase after experiencing water stress

Soil moisture (%)	HS-49xSP-65/32	IP-3AxSP-89/4	IP-3AxSP-65/11	IP-3PxSP-7/5	Jet 1 Agribun
40	16.93 a	12.14 b-d	14.46 ab	9.40 c-f	16.50 a
60	13.27 a-c	11.69 b-d	12.1 b-d	8.77 d-f	11.85 b-d
80	11.08 b-d	8.92 c-f	11.49 b-d	5.95 ef	9.68 c-f
100	10.22 b-e	8.71 d-f	9.74 c-f	5.43 f	8.66 d-f

Note: Numbers with the same letters are not significantly different in the 5% level DMRT test.

During the recovery phase, clones IP-3AxSP-65/11, HS-49xSP-65/32, and Jet-1 Agribun, previously grown on moist soil at field capacity, produced the most fruit, while clone IP-3AxSP-89/4 produced the least (Table 7). The condition of 40% soil moisture for two months caused HS-49xSP-65/32 clone to produce the most fruit during the recovery phase, while the IP-3PxSP-7/5 clone produced the least. In general, clones grown under water stress are able to form lots of fruit during the recovery phase and are more tolerant than clones that produce little or no fruit (Meng *et al*., 2013; Septia *et al*., 2021). Therefore, clone HS-49xSP-65/32 is thought to be more tolerant to water shortages than the other clones.

Table 7 – Amount of fruit harvested (pieces) of some Jatropha clones during the recovery phase after experiencing water stress

Soil moisture (%)	HS-49xSP-65/32	IP-3AxSP-89/4	IP-3AxSP-65/11	IP-3PxSP-7/5	Jet 1 Agribun
40	5.00 a-e	3.33 f	3.83 ef	1.67 g	3.17 f
60	5.16 a-e	4.34 c-f	5.00 a-e	4.00 c-f	4.00 d-f
80	5.17 a-e	4.50 c-f	6.17 ab	4.00 c-f	5.50 a-e
100	5.67 a-c	4.50 c-f	6.50 a	4.67 b-f	5.51 a-d

Note: Numbers with the same letters are not significantly different in the 5% level DMRT test.

Based on the seven observed variables measured, the tested clones produced different indices of stress sensitivity and levels of tolerance to water stress (Tables 8 and 9). The number of fallen leaves during the stress period confirms that the tested clones belong to the very susceptible – very tolerant category, where clone HS-49xSP-65/32 is categorized as a very tolerant clone, while the other clones are categorized as very susceptible (Table 9). Estimates based on the number of fallen leaves during stress also show that clone HS-49xSP-65/32 has a higher tolerance level to water stress than other clones (Table 1).

Table 8 – Stress Sensitivity Index of some Jatropha clones on the seven observed variables and their averages during water stress and recovery

Clones	During water stress			During recovery			
	Number of fallen leaves	Number of leaves formed	Increase in stem volume	Number of fallen leaves	Number of leaves formed	Increase in stem volume	Amount of fruit
HS-49xSP-65/32	9.08	6.01	2.14	10.05	-0.28	1.12	8.48
IP-3AxSP-89/4	1.42	0.15	-1.38	2.88	0.02	-6.81	0.12
IP-3AxSP-65/11	0.83	1.83	17.20	0.49	-0.28	0.30	0.60
IP-3PxSP-7/5	1.39	-69.64	1.43	-122.34	-0.16	0.88	3.90
Jet-1 Agribun	-4.46	2.74	-0.77	1.68	-0.44	14.91	-2.61

Table 9 – Tolerance levels of some Jatropha clones on the seven observed variables and their averages during water stress and recovery

Clones	During water stress			During recovery				Average
	Fallen leaves	Leaves formed	Stem volume	Fallen leaves	Leaves formed	Stem volume	Amount of fruit	
HS-49xSP-65/32	5	5	1	1	5	5	5	3.9
IP-3AxSP-89/4	1	1	1	4	1	5	1	2.0
IP-3AxSP-65/11	1	2	5	5	5	5	1	3.4
IP-3PxSP-7/5	1	1	1	5	5	5	3	3.0
Jet-1 Agribun	1	3	1	5	5	1	1	2.4

Note: 1. Very Susceptible; 2. Susceptible; 3. Moderate; 4. Tolerant; 5. Very tolerant.

From the number of leaves formed during the water stress phase, it can be concluded that clone HS-49xSP-65/32 is very tolerant, Jet-1 Agribun is moderate, IP-3AxSP-65/11 is susceptible, and two other clones are very susceptible (Table 9). Table 2 also shows that HS-49xSP-65/32 has a higher tolerance level than the other clones.

From the increase in stem volume during the water stress phase, it can be concluded that the clones tested were classified as very tolerant – very vulnerable clones. The IP-3AxSP-65/11 clone was very tolerant, while the other clones were classified as very susceptible (Table 9). Table 3 also shows that IP-3AxSP-65/11 has a higher tolerance level than the other clones.

From the number of leaves formed during the recovery phase, it can be concluded that the clones tested were classified as very tolerant – very vulnerable clones. Clone IP-3AxSP-89/4 was very susceptible, while the other clones were very tolerant (Table 9). This result contradicts the initial assumption that the IP-3AxSP-65/11 clone had the lowest tolerance level, and the other clones had the highest (Table 5).

From the amount of fruit formed during the recovery phase, it can be concluded that the clones tested were classified as very tolerant – very susceptible. Clone HS-49xSP-65/32 is very tolerant, clone IP-3PxSP-7/5 is moderate, and the other clones are very susceptible (Table 9). Table 6 also shows that HS-49xSP-65/32 has a higher tolerance level than the other clones.

The differences in the observed variables used to determine the tolerance level of the clones cause the differences in the results obtained. Therefore, determining tolerance levels was carried out by averaging the tolerance level of each observed variable. Thus, the results show that the tested clones are susceptible – tolerant (Table 9). Clone HS-49xSP-65/32 is classified as tolerant, clones IP-3AxSP-65/11 and IP-3PxSP-7/5 are classified as moderate, and clones IP-3AxSP-89/4 and Jet-1 Agribun are classified as susceptible. The response of growth and production of *Jatropha curcas* to the availability of water in the soil is determined by the clones used (Yi *et al*., 2014).

Clone HS-49xSP-65/32 was classified as tolerant to water stress, so this clone was able to suppress leaf loss during stress, increase the number of leaves formed, and increase stem volume, and during the recovery phase, the clone was able to increase stem volume and amount of harvested fruit. Clones IP-3AxSP-65/11 and IP-3PxSP-7/5 were classified as moderate because they were able to suppress leaf loss and accelerate plant growth during the recovery phase.

CONCLUSION

Findings show that the clones tested were classified as vulnerable – tolerant to water stress. Clone HS-49xSP-65/32 is classified as tolerant, clones IP-3AxSP-65/11 and IP-3PxSP-7/5 are classified as moderate, and clones IP-3AxSP-89/4 and Jet-1 Agribun are classified as susceptible.

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