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Selection of some important tobacco genotypes against waterlogging in Indonesia

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Abstract

Stagnant water is an environmental challenge that can have a negative impact on tobacco growth and productivity. Study on waterlogging-tolerant tobacco is still limited. Flood-resistant tobacco genotypes are needed for future agriculture. The aim of this study is to select tobacco genotypes that are tolerant to waterlogging. This study used a factorial randomized block design. The control treatment was 100% waterlogging, with subsequent treatments escalating to 120%, 140%, and 160% (Filed capacity). Waterlogging was given for 75 hours. The observation indicator is the level of wilting of tobacco leaves. Based on the stress index values obtained from the thirty-three tested genotypes, there are three categories of ability to withstand waterlogging, namely, tolerant, moderate and sensitive. Based on the results of the study analysis, it can be interpreted that the Bojonegoro 1 genotype is the only genotype that is included in the category of tolerant to waterlogging. Genotype 1 Bojonegoro was able to survive up to 73 hours with stress sensitivity index score of 0.21.

Keywords: Moderate; Sensitive; Tolerant.
Abbreviations: PGRs_Plant genetic resources.

Introduction

Waterlogging often causes damage to tobacco plants. Tobacco has been used as a raw material for cosmetics, pesticides and medicines (Prasetyo, 2017). Tobacco is usually damaged due to weather anomalies and extreme weather results in tobacco productivity. The high intensity of rain causes the tobacco to lack oxygen supply resulting in root rot. The decline in raw material production is the impact of the extreme weather that has been happening so far (Costa and Farrant, 2019).

Climate change causes high rainfall, sea level rise, which has a negative impact on plant growth and productivity (Raza et al., 2019). Recurring floods on agricultural land in Indonesia cause huge losses in agricultural output. Flood events inducing hypoxic conditions have led to a global reduction of 57% in agricultural output (FAO, 2016)

Waterlogging constitutes a pivotal stressor capable of exerting deleterious effects on plant growth and development, potentially leading to severe growth retardation or mortality (Nguyen et al., 2018). The current investigation underscores the limited exploration of water-tolerant tobacco. Immediate efforts are required to conduct comprehensive studies aimed at developing cultivars resilient to waterlogging, addressing this exigent challenge.

Genetic diversity plays a crucial role in enhancing control mechanisms within ecosystems (Wan et al., 2022). Indonesia hosts a wealth of genetic diversity, with tobacco standing as a key plantation commodity. However, our understanding of the genetic resources associated with water-tolerant tobacco remains significantly limited. Therefore, the collection of genetic resources to obtain desired varieties needs to be undertaken.

Plant genetic resources (PGRs) encompass the heritable material comprising the entire spectrum of alleles inherent in diverse genes within plant species and their wild counterparts. These PGRs constitute the principal reservoir upon which human reliance for future agricultural endeavors is predicated (Salgotra and Chauhan, 2023). Maintaining PGRs is very important for the development and genetic improvement of plant varieties. Currently, the extinction rate of plant species is skyrocketing, and life on Earth is facing its sixth mass extinction event caused by climate change and anthropogenic activity, which could lead to ecological collapse (Salgotra and Chauhan, 2023). This study enhances our comprehension of the repercussions induced by waterlogging. Furthermore, it furnishes valuable insights for the selective breeding of tobacco varieties resilient to waterlogging and optimizing cultivation methodologies.

Result and Discussion

The analysis of variance showed that there was an interaction between genotype and time withstanding the level of waterlogging. This observation indicates that among the tested genotypes, certain genotypes exhibit varying levels of tolerance in comparison to others. Table 1 shows that waterlogging; genotype and their interaction have significant different results. Furthermore, testing with α = 5% showed that the greater the percentage of waterlogging, the shorter the survival time of the tobacco genotype. (Perez-Jimenez et al. 2018). Perez dan Tornero (2021) stated that genotype and inundation resulted in a significant interaction. Thus, from these results, categories of tolerant, moderate, and sensitive genotypes can be determined using the Stress Sensitivity Index (Karuwal et al., 2018).

Table 4 indicates that, there are 3 (three) categories of tobacco genotypes. Bojonegoro 1 genotype is in the tolerant category, genotype K 99, H A 320, Mawar PMK, H 8 94, Semporis, BAT 45, Prancak 95, HN 944, Benyak, T K D + H362, HB 6-15, Coker 176, BB, dan TS 54 + TKD in the moderate category, genotype Kasturi 933, TC 8212, Jowo krempol kerep, Kristian, G T, SGR K 99, B.P, Jimanuk, Jowo ros kerep, K 326, T C ML, Kasturi kraksan, Opot, K 3 19, Merci, TC 918, Kasturi, Sumoris, dan H 362 in the sensitive category.

Lack of oxygen in the soil due to waterlogging is a limiting factor for plant growth and productivity. According to (Rodríguez et al., 2018), Lack of oxygen shifts energy metabolism from aerobic to anaerobic so that it adversely affects the absorption of nutrients and water. As a result, plants show wilting symptoms even when there is plenty of water available. Under conditions of water stress, plants try to withstand water loss by increasing water diffusion resistance, the greater the level of water stress experienced, the higher the water diffusion resistance (Zahra et al., 2021).

Tobacco plants with waterlogged conditions will have an impact on reducing gas exchange between soil and air resulting in reduced oxygen availability for roots, inhibiting oxygen supply for roots and microorganisms. This condition causes hypoxia around the roots. Oxygen plays an important role in metabolic processes that produce energy in cells, so that very low oxygen concentrations in the roots cause disruption of metabolic activity and energy production. This is in accordance with the statement of Colmer and Voesenek (2009) that an increase in inundation of 20% exceeding field capacity causes plants to lack oxygen, causing hypoxia.

The Bojonegoro 1 genotype is the only genotype that is included in the tolerant category with stress sensitivity index score of 0.21. There are 14 genotypes in the moderate category and 18 genotypes in the sensitive category. The GT genotype is the most sensitive genotype among the 33 genotypes with a stress sensitivity index score of 1.46. This is in accordance with the principle of Shelford's Law of Tolerance that "Every organism has an ecological minimum and maximum, namely the lower and upper limits of the organism's tolerance range for environmental factors".

The Bojonegoro 1 genotype at various levels of inundation has the ability to survive the longest compared to other genotypes. The Bojonegoro 1 genotype was able to survive and not wilt for up to 73 hours. On the contrary, the GT genotype has the lowest ability to survive at various levels of inundation compared to other genotypes. The GT genotype only withstood waterlogging for 32.67 hours.

The Bojonegoro 1 genotype comes from an area with an altitude of 156 meters above the sea level. The GT genotype originates from an area with an altitude of 400 meters above sea level. It seems this needs a comprehensive study in the future. Morphologically, the Bojonegoro 1 genotype showed the most adventitious roots compared to the GT genotype.

Root aeration is essential to resist waterlogging (Yamauchi et al., 2020). Root aeration is essential to resist waterlogging (Eysholdt-Derzsó and Sauter, 2017). Dawood et al., (2016), stated that in order to maintain root function in a hypoxic environment, plants form new aerenchymatous adventitious roots, which originate from dormant primordia that have previously formed on the stem. Study result (Sundgren et al., 2018) showed that the tolerant wheat genotype was different from the sensitive genotype. The tolerant genotype developed roots faster during the seedling formation phase and more adventitious roots grew during the inundation treatment. The initial ability to adapt determines the ability to survive from hypoxia in the next phase.

Formation of aerenchyma in the roots is critical to allow diffuse oxygen transport to reach the root tip. In addition,

Table 1. Genotypes used in study.

Genotype name	Location	Height
Kasturi 933	Sampang	300 masl
TC 8212	Blitar	167 masl
Jowo krempol kerep	Bojonegoro	127 masl
K 99	Kediri	199 masl
Kristian	Bondowoso	386 masl
H A 320	Malang	500 masl
Mawar PMK	Jember	400 masl
G T	Jember	400 masl
SGR K 99	Jember	300 masl
B.P Jimanuk	Blitar	167 masl
Jowo ros kerep	Bojonegoro	156 masl
K 326	Jember	400 masl
T C ML	Jember	300 masl
Bojonegoro 1	Bojonegoro	156 masl
H 8 94	Jember	400 masl
Semporis	Bodowoso	345 masl
Kasturi kraksan	Probolinggo	35 masl
Opot	Pamekasan	250 masl
K 3 19	Bojonegoro	127 masl
BAT 45	Situbondo	500 masl
Prancak 95	Pamekasan	50 masl
HN 944	Jember	600 masl
Merci	Probolinggo	35 masl
Benyak	Bondowoso	240 masl
T K D + H362	Jember	400 masl
HB 6-15	Jember	400 masl
Coker 176	Bondowoso	240 masl
TC 918	Jember	600 masl
BB	Probolinggo	35 masl
Kasturi	Jember	400 masl
Sumoris	Bondowoso	345 masl
H 362	Jember	400 masl
TS 54 + TKD	Jember	400 masl

longitudinal oxygen transport towards the root tip can be hampered if there is excessive radial loss of oxygen to the rhizosphere. In this regard, auxin contributes to the formation of ethylene-dependent aerenchyma in roots (Yamauchi et al., 2020). In the roots of upland plants, such as maize (*Zea mays* ssp. mays) and wheat (*Triticum aestivum*), lysogen aerenchyma is generally not formed under aerobic conditions, but its formation is induced under oxygen deficient conditions (Pedersen et al., 2021b).

During the post-waterlogging period, willaw plants showed increased adventitious root production and stomatal conductance (Mozo et al., 2021). Tomato plants are susceptible to flood stress. Several adaptive responses help reduce the damaging effects of hypoxia on roots. The signal for decreasing O_2 seems to control the interacting puddle time periods (Meitha et al., 2018). In addition, a hypoxic transition phase naturally occurs for some plant storage tissues or organs (Cukrov, 2018; Xiao et al., 2018). Ethylene-mediated aerenchyma formation, stem hypertrophy, and adventitious root formation facilitate O_2 transport and may act as release mechanisms that allow hypoxia tolerance (Mignolli et al., 2020). Genes encoding proteins with potential roles in aerenchyma formation have been identified using an RNA-Seq approach in tomato roots under hypoxia (Safavi-Rizi et al., 2020).

Table 2. ANOVA of genotype survival times.

Source of diversity	db	JK	KT	F Count	F Table		Nilai P
					5%	1%	
Pool	2	12,717.66	6,358.83	59.62**	5.14	10.92	0.0001
Replication	6	639.92	106.65	1.94^{tn}	2.15	2.90	0.0760
Genotype	32	153,344.75	4,792.02	87.24**	1.50	1.77	0.0000
Genotype*pool	64	29,062.57	454.10	8.27**	1.38	1.57	0.0000
Error	192	10,546.08	54.93				
Total corrected	296	206,310.97					
kk = 8.90%							

Table 3. Further test results for various waterlogging treatments for 33 tobacco genotypes.

Genotype	Waterlogging 120%	Waterlogging 140%	Waterlogging 160%	Average Genotype
Kasturi 933	64.33^{hi}	60.00^{hijk}	61.33^{fghij}	61.89^{hi}
TC 8212	67.67^{ghi}	58.67^{ijk}	58.00^{ghij}	61.44^{hi}
Jowo krempol kerep	86.00^{efgh}	83.67^{efgh}	78.00^{defgh}	82.56^{ef}
K 99	86.00^{efgh}	85.33^{defg}	84.33^{cdef}	85.22^{ef}
Kristian	67.67^{ghi}	61.00^{ghij}	60.67^{fghij}	63.11^{hi}
H A 320	90.00^{defg}	82.67^{fghi}	80.00^{defgh}	84.22^{ef}
Mawar PMK	88.67^{efgh}	84.33^{efgh}	80.67^{defgh}	84.56^{ef}
G T	59.67^{i}	30.67^{l}	32.67^{kl}	41.00^{j}
SGR K 99	68.00^{ghi}	63.33^{fghij}	60.67^{fghij}	64.00^{hi}
B.P Jimanuk	67.00^{ghi}	63.67^{fghij}	61.00^{fghij}	63.89^{hi}
Jowo ros kerep	68.00^{ghi}	68.67^{fghi}	62.33^{fghij}	66.33^{ghi}
K 326	68.67^{ghi}	64.67^{fghij}	61.00^{fghij}	64.78^{hi}
T C ML	64.33^{hi}	67.00^{fghi}	62.00^{fghij}	64.44^{hi}
Bojonegoro 1	161.67^{a}	155.67^{a}	151.00^{a}	156.11^{a}
H 8 94	86.00^{efgh}	83.67^{efgh}	81.00^{defgh}	83.56^{ef}
Semporis	102.67^{bcdef}	107.33^{bcde}	64.33^{fghij}	91.44^{de}
Kasturi kraksan	103.00^{bcdef}	65.67^{fghi}	50.67^{ijkl}	73.11^{fgh}
Opot	108.00^{bcde}	61.33^{ghij}	57.00^{hijk}	75.44^{fgh}
K 3 19	83.67^{efghi}	81.67^{fghi}	80.67^{defgh}	82.00^{ef}
BAT 45	89.33^{defg}	85.00^{efg}	79.33^{defgh}	84.56^{ef}
Prancak 95	116.67^{b}	120.00^{b}	108.33^{bc}	115.00^{b}
HN 944	115.67^{bc}	109.67^{bcd}	108.33^{bc}	111.22^{bc}
Merci	83.00^{fghi}	80.33^{fghi}	83.67^{def}	82.33^{ef}
Benyak	116.67^{b}	113.00^{b}	70.67^{efghi}	100.11^{cd}
T K D + H362	114.67^{bc}	112.67^{b}	45.67^{jkl}	91.00^{de}
HB 6-15	118.00^{b}	112.67^{b}	96.67^{bcd}	109.11^{bc}
Coker 176	117.67^{b}	114.00^{b}	111.67^{b}	114.44^{b}
TC 918	70.33^{ghi}	86.33^{cdef}	82.00^{defg}	79.56^{efg}
BB	117.67^{b}	110.67^{bc}	111.33^{b}	113.22^{bc}
Kasturi	70.33^{ghi}	74.33^{fghi}	77.00^{defgh}	73.89^{fgh}
Sumoris	90.00^{defg}	40.67^{jkl}	30.33^{l}	53.67^{ij}
H 362	91.33^{cdefg}	35.67^{kl}	60.67^{fghij}	62.56^{hi}
TS 54 + TKD	113.67^{bcd}	112.67^{b}	94.33^{bcde}	106.89^{bc}
HSD 5%	24.47	24.47	24.47	14.13
Location Average	91.39^{a}	82.93^{b}	75.37^{c}	

Notes: Numbers followed by the same letter in the same column are not significantly different based on the BNJ level test 5%

Materials and methods

This study was carried out in the Greenhouse of PGRI Argopuro University Jember, East Java Indonesia from June to August 2023.

Study materials

The planting material comprised of 33 tobacco genotypes, specifically: Bojonegoro 1, K 99, H A 320, Mawar PMK, H 8 94, Semporis, BAT 45, Prancak 95, HN 944, Benyak, T K D + H362, HB 6-15, Coker 176, BB, and TS 54 + TKD, Kasturi 933, TC 8212, Jowo krempol kerep, Kristian, G T, SGR K 99, B.P, Jimanuk, Jowo ros kerep, K 326, T C ML, Kasturi kraksan, Opot, K 3 19, Merci, TC 918, Kasturi, Sumoris, and H 362. The cultivation employed regosol soil with a pH of 5.8, and each polybag contained 19 kg of soil.

Table 4. Sensitivity status of 33 tobacco genotypes to waterlogging for 1 week.

No	Genotype	Score Stress Sensitivity Index	Status
1	Kasturi 933	1.23	Sensitive
2	TC 8212	1.24	Sensitive
3	Jowo krempol kerep	1.01	Sensitive
4	K 99	0.98	Moderate
5	Kristian	1.22	Sensitive
6	H A 320	0.99	Moderate
7	Mawar PMK	0.99	Moderate
8	G T	1.46	Sensitive
9	SGR K 99	1.21	Sensitive
10	B.P Jimanuk	1.21	Sensitive
11	Jowo ros kerep	1.18	Sensitive
12	K 326	1.20	Sensitive
13	T C ML	1.20	Sensitive
14	Bojonegoro 1	0.21	Tolerant
15	H 8 94	1.00	Moderate
16	Semporis	0.91	Moderate
17	Kasturi kraksan	1.11	Sensitive
18	Opot	1.08	Sensitive
19	K 3 19	1.01	Sensitive
20	BAT 45	0.99	Moderate
21	Prancak 95	0.65	Moderate
22	HN 944	0.69	Moderate
23	Merci	1.01	Sensitive
24	Benyak	0.82	Moderate
25	T K D + H362	0.92	Moderate
26	HB 6-15	0.72	Moderate
27	Coker 176	0.66	Moderate
28	TC 918	1.04	Sensitive
29	BB	0.67	Moderate
30	Kasturi	1.10	Sensitive
31	Sumoris	1.32	Sensitive
32	H 362	1.23	Sensitive
33	TS 54 + TKD	0.74	Moderate

Notes : ISC < 0,5 Tolerant, 0,5 < ISC < 1,0 Moderate. dan ISC > 1,0 Sensitive (Karuwal *et al*., 2018).

Treatment of hypoxia stress

This study employed a factorial randomized block design comprising of three replications and four distinct treatments. The treatments given were 100% waterlogging as control, 120%, 140%, and 160% (in term of field capacity). A total of 33 tobacco plant genotypes were observed. The materials needed in this study are polybags measuring 50 x 50 cm, and buckets. Waterlogging is provided for 75 hours.

Seeds from thirty-three distinct tobacco varieties were sown in tray pots, and after 40 days, the resulting tobacco seedlings were transplanted into polybags. The waterlogging treatment was initiated 45 days post-planting. Hourly observations were conducted over the course of one week, focusing on the wilting levels of tobacco leaves as the observational indicator.

Data collection and analysis

Analysis of variance (ANOVA) and the follow-up test of significant differences (BNJ) with α=5% were analyzed using PKBT-STAT 3.1 (http://pbstat.com/pkpt-stat/index.php). Furthermore, the tolerant, moderate and sensitive categories were determined based on the stress sensitivity index (ISC) (Karuwal et al., 2018) if the ISC value < 0.5 then the plant is tolerant to stress, if 0.5 < ISC < 1.0 then the plant is classified as moderate or between tolerant and sensitive to stress, and if ISC > 1.0 then the genotype is sensitive to stress. The ISC formulation used is based on Fisher and Maurer (1978):

$$\mathrm{ISC} = \frac{(1 - \frac{\mathrm{Ys}}{\mathrm{Yp}})}{(1 - \frac{\acute{\mathrm{Y}}\mathrm{s}}{\acute{\mathrm{Y}}\mathrm{p}})}$$

Notes : ISC = Stress sensitivity index; Yp = observed value of each genotype under control conditions; Ys = observed values of each genotype under stress conditions. Ýp = average value of all genotypes under normal conditions. Ýs = average value of all genotypes under stress conditions. The ISC is calculated based on the wilting speed of the plant, the faster the wilting, the greater the ISC value, the longer the wilting, the smaller the ISC value.

Conclusion

Tobacco genotypes characterized as intolerant exhibit a series of leaf abscissions, culminating in complete wilting. Subsequently, the leaves undergo a gradual yellowing process from the bottom to the top. Following the yellowing phase, premature aging of the leaves ensues, ultimately resulting in leaf necrosis. The study's findings and genotype selection identified three distinct tobacco genotypes with varying responses to hypoxia. Specifically, the Bojonegoro 1 genotype demonstrated tolerance, the Benyak genotype exhibited intermediate sensitivity, and the GT genotype displayed sensitivity to hypoxic conditions.

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