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Analysis of Tobacco Physiology Deli Tolerance Drought

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ABSTRACT

Physiology character can show a tolerant mechanism of drought. The objective of this experiment is to find physiology character of the tolerant and sensitive drought of Deli tobacco. Six tobacco varieties were selected namely (K-399 (tolerant), Connecticut x Lokal (tolerant), PVH-50 (tolerant), DB-101 (sensitive), VDM-2 (sensitive) and Deli-4 (control). The plants were exposed to water stress by watering them every day and not watering since 30 days after planting. Results of the experiment indicated that higher proline can be used as the indication of drought tolerant of tobacco.

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INTRODUCTION

Deli tobacco plants that respond to drought stress have been obtained from previous studies, namely varieties K-399 and PVH-50 as well as strains Connecticut x Local, was selected as tolerant candidates, while varieties DB 101, and VDM-2 as sensitive candidates. Tolerance and sensitivity to drought occur because of a mechanism in plants that results in differences in the ability to grow under restricted water conditions.

The tolerance mechanism of the plant to drought stress varies depending on its genetic ability (Pirdahsti *et al*., 2004). The classification of drought stress resistance is divided into 3 namely : (1) release to drought stress, that is the ability to complete its life cycle before experiencing severe water deficit as indicated by the development of rapid flowering system and the development of tissue plasticity (2) tolerance with high tissue water potential, that is the ability of plant to maintains tissue potential by increasing water absorption or suppressing water loss (plants have the ability to increase root system, stomatal regulation, reduction of radiation absorption by wax formation, thick coat and decrease of surface evapotranspiration by leaf narrowing and leaf fall) and (3) Tolerance of low tissue water potential, i.e. the ability of plants to maintain cellular turgor pressure through the accumulation of solutes such as sugars and amino acids and by increasing cell elasticity.

To maintain turgor pressure, a number of biochemical characters can be used to distinguish the tolerance levels of plants against drought stress. Some research indicates that the mechanism of plant adaptation to overcome drought stress is by regulating the osmotic potential of the cells by increasing the synthesis of certain compounds. In such mechanism, there is synthesis and accumulation of organic compounds that can decrease the osmotic potential, thus decreasing the water potential in cells without limiting the function of enzymes and cell turgor. Some of the compounds that play in the osmotic adjustment of the cells include osmotic sugars (Wang *dkk*. 1995: Yakushiji *dkk.*, 1998), proline and betaine (Maestri *et al*., 1995), dehydrin protein (Close, 1997) and abscisic acid (ABA) which accelerate accumulation of these compounds (Dingkuhn *et al*., 1991). Candidate for osmotic adjustment to drought stress that has been widely used as drought tolerant criteria is proline (Delauney and Verma, 1993). Higher proline content has been found in coffee plants that are relatively resistant to drought stress (Maestri *et al*., 1995).

For Deli tobacco, no one has reported any major compounds related to plant tolerance to

drought stress. In this study, several compounds suspected to have links to the tolerance was tested, namely total amino acids, proline, and total sugar.

MATERIALS AND METHODS

Polybag experiments were conducted in the greenhouse of Deli Tobacco Research Center, Medan, North Sumatra.

Plant materials used were 6 varieties/ strains of tobacco as selected collections of BPT, result of previous research, namely 3 tolerant varieties/strains such as Connecticut x Local, K-399 and PVH-50 and sensitive varieties/strains such as DB-101, VDM -2 and Deli-4 obtained from collection of Deli Tobacco Research Center, Medan and Tobacco and Fiber Research Center, Malang.

In the implementation, plant material that is 40-days-old seedlings are kept in the greenhouse of Deli Tobacco Research Center, Deli Serdang. Seedlings are then removed and maintained in a 25 kg polybag using topsoil medium.

The experiment is separate plot design, two factors, and three repetitions. The first factor is the water supply consists of water every day and not water from 30 days after planting. The second factor is varieties consisting of six varieties namely Connecticut x Local, DB-101, PVH-50, K-399, Deli-4, and VDM-2.

Treatment of Drought Stress

Treatment of drought stress was done at 30 days after planting that is the long days without watering that is 7 days and 14 days. As controls, plants watered daily in accordance with plant requirement.

Data Observation and Analysis

The content of proline, amino acid, total sugar, N, P, K, and Mg was tested at the age of 37 days after planting (7 days after not watering) and 44 days after planting (14 days not watered). Irigoyen et al. (1992) in Sudarsono and Widoretno (2003) carried out proline analysis of leaf tissue according to the method of Bates et al. (1973) in Hamim et al (1996), amino acids by the method of Ninhydrin (1984) in Karyudi (1999) and total sugar.

RESULTS AND DISCUSSION

3.1 Proline Content (μ mol/g dry weight)

The results showed that the varieties tested had very different responses to the drought stress in producing proline on the leaves. The results of proline measurement of tobacco leaf of various varieties and strains are presented in Table 1.

The results showed that there was an increase in proline content measured in the drought stress treatment for 7 days and 14 days in all tested varieties, but the increase percentage was different. The tolerant variety (K-399, Connecticut x Local, PVH-50) has higher proline content compared to sensitive varieties (DB 101, Deli-4 and VDM-2) in drought stress conditions. The tolerant strain, Connecticut x Local, has proline content up to 275.30% of control after not watering for 7 days and 436.55% after not watering for 14 days. Varieties K-399 have proline content up to 233,33% compared to control after not watered for 7 days and 430,49% after not watered for 14 days. Varieties PVH-50 have proline content up to 232,77% after not watered for 7 days and 428,57% after not watered for 14 days. Conversely, sensitive varieties, DB-101, have 146.12% of proline content after not watered for 7 days and 196.71% after not watered for 14 days. The VDM-2 have 116.96% of proline content after not watered for 7 days and 178.54% after not watering for 14 days. Deli-4 have 126.09% of proline content after not watering for 7 days and 196.42% after not watering for 14 days.

At the drought stress for 7 and 14 days, the highest proline content found at strain Connecticut x Local, that is 6.80 μ mol g/dw and 10.87 μ mol g/dw, which was significantly different from the proline content of DB 101, VDM-2 and Deli -4, whereas with varieties K-399 and PVH-50 is not significantly different. Varieties Deli-4, DB-101, and VDM-2 were not significantly different either.

3.2 Total Sugar Content (μg / g DW)

The results of analysis of variance showed that the varieties tested had no significant response to drought stress in producing sugar in tobacco leaf. The results of sugar content measurements of tobacco leaf of various varieties and strains are presented in Table 1.

The results of the measurements showed that there were variations in total sugar content among observed varieties and strains due to the genotype difference. Almost all of the

varieties showed an increase in sugar content in the presence of drought stress, except DB-101 and Deli-4 that showed decreased sugar content after experiencing drought stress for 7 days. Varieties K-399 contained sugar content of 106.38% and 101.21% from controls for drought stress for 7 days and 14 days respectively. Strain Connecticut x Local has a sugar content of 119.27% and 108.00% for drought stress for 7 days and 14 days respectively. Varieties PVH-50 contained sugar content of 134.50% and 114.69% in drought stress for 7 days and 14 days respectively. DB-101 varieties have a sugar content of 85.64% and 111.06% in drought stress for 7 days and 14 days respectively. Varieties of VDM-2 have a sugar content of 101.18% and 127.95% on drought stress for 7 days and 14 days respectively. Finally, Deli-4 variety has a sugar content of 94.81% and 113.26% on drought stress for 7 days and 14 days respectively.

The varietal response was not significantly different from drought stress, but the highest sugar content was found at PVH-50, followed by Connecticut x Local, VDM-2, K-399, Deli-4 and DB-101 after severe drought stress for 7 days. After experiencing drought stress for 14 days, the highest sugar content was found at VDM-2, followed by PVH-50, DB-101, Deli-4, Connecticut x Local, and K-399.

3.3 Amino Acid Content (mM)

The results of analysis of variance showed that there are very different responses to the drought stress in producing amino acids on the leaves after drought stress for 14 days, but after drought stress for 7 days did not show any significant difference in response. The results of amino acid content measurements of tobacco leaves from various varieties and strains of tobacco are presented in Table 1.

All varieties, both sensitive and tolerant, showed increased amino acid content compared to the controls. After experiencing a drought stress for 7 days, K-399 have amino acid content of 198.07% compared to control, Connecticut x Local 202.72%, PVH-50 200.83%, DB-101 159.08%, VDM -2 156.34% and Deli-4 158.31%.

After experiencing drought stress for 14 days, K-399 have amino acid content 248.62% compared to control, Connecticut x Local 253.69%, PVH-50 239.23%, DB-101 176,65%, VDM -2 170.35% and Deli-4 173.61%.

The highest amino acid content after 14 days of drought stress was found at Connecticut x

Local, which very significant different with DB-101, Deli-4, and VDM-2, but not significantly different with PVH-50 and K-399. Varieties DB-101, Deli-4, and VDM-2 showed no significant difference.

Table 1: Mean of Proline content, Total Sugar and Amino Acid for 6 Varieties and Deli Tobacco Strains on Two Treatments of Drought Stress

Treatment	Proline content				Total Sugar				Amino Acid			
	7 days	%	14 days	%	7 days	%	14 days	%	7 days	%	14 days	%
Application												
Interval	2,36		2,43		146,		149,		232,		238,58	
I_0	4,49		7,60		46		45		20		505,58	
I_1					155,		168,		417,			
Variety	4,05		6,53		53		26		92		428,91	
V_1	4,64		6,68								447,30	
V_2	3,96		6,48		145,		149,		356,		417,21	
V_3	2,86		3,61		41		45		64		323,56	
V_4	2,43		3,25		153,		157,		373,		303,62	
V_5	2,60		3,55		50		77		58		311,88	
V_6					158,		164,		352,			
Interaction	2,43		2,46		90		29		76		246,06	
I_0V_1	cB		cB		142,		162,		300,		cC	
I_0V_2	2,47		2,49		06		94		01		252,93	
I_0V_3	cB		cB		153,		163,		281,		cC	
I_0V_4	2,38		2,45		95		17		15		245,97	
I_0V_5	cB		cB		152,		155,		286,		cC	
I_0V_6	2,32	233,	2,43	430,	15	106,	52	101,	25	198,	233,91	248,
I_1V_1	cB	33	cB	49		38		21		07	cC	62
I_1V_2	2,24	275,	2,33	436,	140,	119,	148,	108,	239,	202,	224,61	253,
I_1V_3	cB	30	cB	55	91	27	55	00	30	72	cC	69
I_1V_4	2,30	232,	2,40	428,	140,	134,	151,	114,	246,	200,	227,97	239,
I_1V_5	cB	77	cB	57	01	50	70	69	81	83	cC	23
I_1V_6	5,67	146,	10,59	196,	135,		153,	111,	234,	159,	611,76	176,
	bA	12	aA	71	52	85,64	05	06	52	08	aA	65
	6,80	116,	10,87	178,	153,		154,	127,	231,	156,	641,67	170,
	aA	96	aA	54	05	101,	40	95	60	34	aA	35
	5,54	126,	10,50	195,	153,	18	143,	113,	219,	158,	588,44	173,
	bA	09	aA	42	05		16	26	35	31	aAB	61
	3,39		4,78		156,	94,81	145,		221,		413,21	
	cB		bB		20		85		63		bBC	
	2,62		4,16		149,		150,		473,		382,63	
	cB		bcB		90		35		98		bC	
	2,90		4,69		166,		163,		500,		395,79	
	cB		bB		99		84		34		bC	
					182,		175,		470,			
					27		53		99			
					131,		171,		368,			
					07		48		42			
					154,		183,		342,			
					85		17		94			
					148,		165,		350,			
					10		19		87			

DISCUSSION

The increase in leaf proline content of the sixth tobacco varieties with increasing of drought stress (Table 1) is consistent with the finding of previous studies, but tolerant varieties have higher proline content (% of control).

In plants that are more tolerant to drought stress, the proline content found higher than intolerant ones. The increasing of proline content in tobacco leaf of tolerant varieties on drought stress is confirming the previous studies, which also reported that there is a higher proline content in leaves of some drought-tolerant varieties of plants. This is due to lower leaf water potential of the tolerant variety. There is a close relationship between the decrease in osmotic potential and the increase in free proline content of leaves. Varieties that have high proline content also have lower osmotic potential. According to Kirkham (1990), proline plays to decrease leaf water potential by using osmotic potential.

Drought stress in tobacco plants causes cells dehydration which characterized by leaf staining due to decrease in leaf water potential. The logical consequence of decreasing the cells water volume is the increased concentration of dissolved compounds and changes in the osmotic potential value of the cell. Increasing the concentration of the dissolved compound is followed by the accumulation of osmotic compounds in response to drought stress, decreasing the osmotic value of the cell. The decrease in the osmotic potential value of the cell occurs as the counterweight to the decline in the potential value of the leaf water so that cell turgidity can be preserved to prevent the occurrence of cell membrane damage and plasmolysis. This mechanism is known as osmotic adjustment and the compound that plays in the osmotic adjustment is called as the osmotic compound.

According to Kirkham (1990), proline plays in the maintenance of cell turgor that is as a counterweight to the decrease of leaf water potential by lowering the osmotic potential although the leaf water potential remains low. Thus, allowing stomata opening to allow carbon dioxide diffusion in the process of photosynthesis. Low water potential results in closing stomata, to reduce transpiration but inhibit the photosynthesis and another metabolism. Dingkuhn *et al*. (1991) in rice found that despite differences in proline accumulation among cultivars, but the proline was negatively correlated with water potential and positively correlated with osmotic adjustment. This means that plants more capable of adjusting cosmetics are better to adapt to the negative effects of stress.

Proline accumulation in tobacco leaf that experiencing drought stress is 436.55% from control. This finding has been reported in several previous studies. According to Maggio *et al*. (2002), amino acids accumulation is an active process associated with drought stress and proline is one of amino acid that most actively accumulated. The rate of proline synthesis can be increased 10 times in adaptive tomato cell cultures to drought stress. In *Brassica napus*, proline content can be increased up to 68 times due to drought stress.

The difference of proline content among the tested tobacco varieties shows the difference of plant response to drought stress, which is the genetic characteristics of the plant. Singh *et al*. (1993) reported that when 10 barley cultivars were treated with drought stress at certain intervals, the ability to accumulate proline was positively correlated with the stability of the yields on dry soil. The differences in proline levels reflect differences in genetic control on metabolic responses at the same level of internal water stress as a function of genetic control differences in overcoming internal drought stress.

The phenomenon of increased proline levels is a common symptom when plants are experiencing stress, especially drought and salinity (Delauney and Verma, 1993; Yoshiba *et al*., 1997; Minarsih *et al*., 1998), and this is an indication of positive correlation with plant adaptation (Delauney and Verma, 1993). By considering differences in proline levels and the evaluation of sensitive and tolerant plants on stress, some researchers have suggested that these can be considered as germplasm selection criteria for drought stress tolerance and salinity (Delauney and Verma, 1993; Kuznetsov and Shevyakova, 1997; Yoshiba *et al*., 1997; Minarsih *et al*., 1998).

Proline accumulation in plants with drought stress is caused by activation of proline biosynthesis and inactivation of proline degradation (Nambara *et al*., 1998). Kishor *et al*. (1995) reported that transgenic tobacco plants produce more proline than controls. Increased proline content in plants experiencing drought stress is caused by proline biosynthesis, which includes the process of protein hydrolysis and oxidative degradation.

The results of the association between gene expression for enzymes involved in biosynthesis and proline metabolism and accumulation in drought stress conditions indicate that the level of proline in plants is regulated at the transcription level (Yoshiba *et al*., 1997). Barrnet and Nailor (1966) in Hamim (1995) suggest many researchers claim that many free prolines are accumulated as the response to observable water stress on leaves that are still attached or that

have fallen on many cultivated plants.

Increased proline content in leaf plants that suffer from stress is because the proline has a role related to resistance to drought stress. The role of proline is not only limited to osmotic adjustments associated with water status but also has other roles such as neutralizing the toxic effects of NH_3 from protein hydrolysis, as an energy source and a source of N for the recovery of physiological processes of after stress. It is also known that the adaptation response to stress does not work alone but involves many other factors (Kirkham, 1990). Proline accumulation is thought to be closely related to the proline ability to act as an osmoregulator, as a protective agent for cytoplasmic enzymes and membrane enzymes or as the storage material for growth after the plant is under stress (Maggio *et al*., 2002).

Testing of sugar content is also performed on tobacco leaf. In general, sugar content increased after experiencing drought stress. Increased sugar in plants suffering from drought stress due to the function of sugar in maintaining bilayer membrane stability and protecting proteins (Darbysire, 1994). Gerbre *et al*. (1997) reported the accumulation of glucose and fructose in *Populus deltoid*s can decrease leaf osmotic potential to help maintain the turgidity under water stress conditions. However, some studies show the contrary result on the effect of drought stress on sugar accumulation on wheat crops. Several studies have reported that the sugar content increases (Jones and Turner 1981, Munns & Weir, 1979), or decreases (Hanson & Hitz, 1982) or constant (Morgan, 1992).

In this study, not all varieties have increased the sugar content after experiencing drought stress, so the sugar cannot reflect the relationship with drought stress. Sugar undergoes dynamically metabolism and translocation. According to Watanabe *et al*., (2000), the difference in total sugar content of leaves is actually associated with differences in growth among species rather than with drought stress. Kirkham (1990) states under drought stress, plants respond by increasing the level of sucrose. Increased sucrose was found in sugar beet (Dubey, 1996), cherry (Yakushiji *et al*., 1998), apples (Wang *et al*., 1995), but in sorghum, there was no difference in sucrose levels between the drought tolerant and intolerant. This study found that there was an increase in sugar content intolerant and sensitive varieties, except for K-399. Based on the contradictory phenomenon, it is very difficult to draw a general conclusion using sugar as characteristic of a plant tolerant or sensitive to drought stress, although sugar plays in osmotic adjustment at the time of the plants suffer from osmotic stress especially in chloroplast where photosynthesis produce the dissolved

compound.

Varietal responses to changes in osmotic sugars show that drought stress does not significantly affect changes in sugar levels; there is only a tendency to increase in sugar content. According to Yu (1999), plants that suffer drought stress will decrease photosynthesis due to low photosynthetic rate or even stopping so that the plant is at the level of sugar starvation. Carbohydrate deficiency can result in inhibiting cell growth, degraded carbohydrate reserves, and decreased respiratory rate.

CONCLUSION

Tolerance mechanisms of Deli tobacco for drought stress can be analyzed from leaf proline content. The tolerant variety has the ability to increase the proline content higher than the sensitive varieties so it is an important compound to demonstrate tobacco plant tolerance to drought stress.

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