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The Dependence of *N*-Malonyltryptophan Formation in Plants on Water Deficit (Review)

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ABSTRACT

Drought stress in plants is accompanied by several metabolic changes. One of them is the appearance of *N*-malonyltryptophan (MT) during leaf wilting of many species, but there is a significant number of plant species in which the appearance of MT did not occur. Plants of some species were able to synthesize also *N*-acetyltryptophan (AT). Excised tomato leaves incubated with D-amino acids (including D-Trp) transform them into malonyl- and acetyl-derivatives even without water deficit. However, MT which appeared during water deficit has been shown to contain L-Trp. Amino acid—1-amino-cyclopropane-1-carboxylic acid (ACC) is also malonylated during water deficit, but other L-amino acids were not malonylated. *N*-malonyl transferases specific for Trp and ACC have been found in several plants. The existence of *N*-malonyltransferase specific to L-Trp and appeared during water deficit in plants forming MT is supposed, but clear experimental proof has not been obtained yet. Plants can transform MT applied exogenously into Trp and further to indole-3-acetic acid (IAA). But no evidence has been appeared up to now that endogenous MT may be a source of IAA. It is unknown till now why it is necessary for plants of many species to malonylate only Trp during water deficit. How MT metabolized in animals and if it affects them is also unknown. The necessity to use molecular-genetic approaches for the elucidation of the physiological significance of MT formation during water deficit is underlined.

1. INTRODUCTION

1.1. The Role of Tryptophan in Plants

Trp is one of 20 amino acids participating in protein synthesis and it is very important for plants. The

dependence of plant live on Trp content is illustrated with their high sensitivity to 5-methyl-Trp—an antimetabolite of Trp [1]. Schallenberg and Berlin [2] found that cells of *Catharanthus roseus* resistant to 5-methyl-Trp had up to 30 times the normal levels of free Trp. It is essential for plants and they must regulate their content effectively.

Trp is the precursor of IAA, melatonin (AMT), glucosinolates and other substances which participate in growth and development regulation and the resistance to drought stress. Lee *et al.* [3] reported that gene *YUCCA7* which participated in IAA synthesis in Arabidopsis enhanced drought resistance. AMT increased the plant resistance to some abiotic and biotic stresses: drought, cold, chemical, salt, fungi and bacteria. It caused also positive effects on the crop productivity [4, 5], In *Brassicaceae*, drought stress resistance was increased due to the induction of significant accumulation of Trp metabolite glucosinolates (cited from [6]), and it may be supposed to be a consequence of the increased Trp content.

Trp content in plants is usually low when compared with the contents of other amino acids. It amounts to 1 - 10 *η*moles per 1 g fw in growing vegetative plant parts [1, 7], whereas other amino acids such as Asp, Glu, Pro and Ala are contained in quantities of 100 - 1000 *η*moles/mg fw [1, 8].

1.2. The Regulation of Tryptophan Content in Plants

Plants regulate Trp content using changes in the synthesis rates of Trp, proteins, IAA and other its metabolites Trp is synthesized in chloroplasts with the participation of indolyl-3-glycerophosphate and serine [9]. Trp transformation is irreversible when its molecule is modified significantly, such as during IAA, alkaloid, serotonin and melatonin syntheses, but may be released from its binding with other amino acids during protein synthesis, and conjugation with low-molecular substances [10-12]. and even with IAA [13]. Reversible conjugations suggest that under changeable environmental, developmental or physiological conditions these compounds can be a source of free Trp.

It is possible that other ways of Trp transformation may exist: oxidation, transformation of L-Trp to D-Trp [14-17] and plants have suitable enzyme for this—tryptophan racemase [18, 19]. Trp is used for glucobrassicin [20] and AMT [4, 5] syntheses.

Physiological regulation of Trp content includes its intracellular compartmentation and transportation to different plant parts.

1.3. Tryptophan Malonylation as a Means for Its Metabolyzation

MT was found in the experiments of Good and Andreae [21] as a product of the metabolism of Trp infiltrated into plants. They were the first investigators which found MT in plants. Their study of the effect of exogenous Trp on the synthesis of IAAsp in excised pea epicotyls revealed that the unknown indolic substance appeared which was very similar to IAAsp by chromatographic properties in paper chromatography and color reaction with Ehrlic reagent, but had different color with Salkowski reagent. Its hydrolysis with $Ba(OH)_2$ yielded Trp, but not IAA as may be expected in the case of IAAsp. They resumed that this substance is MT. Working further; they found MT presence in field grown tomato, spinach, pea and oats plants which were not treated with Trp. It is remarkable that Rekoslavskaya and Gamburg [22] also found MT trying to search endogenous IAAsp in tomato seedlings. They observed also difference of contained in tomato seedlings substance from IAAsp by color reaction with Salkowski reagent and the release of Trp instead of expected IAA during MT hydrolisis.

Malonylation of D-Trp may be considered as a particular case of the ability of plants to malonylate many (but not all) exogenous D-amino acids [14, 23, 24] in order to prevent their participation in plant metabolism, so as D-amino acid oxidase eliminate them [25]. One other amino acid which also undergoes malonylation is 1-aminocyclopropane-1-carboxylic acid (ACC)—an ethylene precursor [26, 27].

MT was found as an endogenous compound in plants [21, 28-35] suggesting that endogenous MT contained D-Trp. Its presence in plants has long been considered as the evidence of plant's ability to synthesize D-Trp. However, it was established that endogenous MT in wilted leaves, seeds, seedlings, tissue cultures [30], young tomato leaves, male ginkgo flowers (pollen cone), coconut milk [13], and fruits of the

South-American plant *Pithecellobium dulce* (Roxb.) Benth. [36] contained L-Trp.

It was observed that MT formed and accumulated in fruits, seeds and seedlings [13, 29, 30, 36-38] as well as in wheat sseedling's roots [28] and *in vitro* cultured cells [19, 30, 37]. It is evident that other physiological processes in plants were also accompanied by MT formation and accumulation.

2. MALONYLTRYPTOPHAN APPEARANCE DURING WATER DEFICIT

2.1. First Observations

The appearance of MT in wilted tomato (*Solanum lycopersicum* L.), potato (*Solanum tuberosum* L.) and wheat (*Triticum aestivum* L.) leaves was shown by Rekoslavskaya and Gamburg [32]. It was accompanied by an increased L-Trp content. All this became a reason for the study of the formation of MT during leaf wilting. These results have been confirmed in a publication by of Liu *et al.* [39]. It was shown that the sum of free amino acids in tomato leaves increased approximately 10 times due to wilting [40]. The content variation was different among amino acids: content of Ala decreased, Asp, Glu, Gly, Cys, Met—increased less than 10 times, Pro, Val, ILe, Leu, Tyr, Trp, His, Lys—increased to more than 10 times and Phe and Arg increased to more than 100 times (Table 1).

The contents of all extracted amino acids did not decrease after their treatment with D-amino acid oxidase from pig kidneys, what evidenced on absence of free D-amino acids in wilting tomato leaves [40].

Table 1. Effect of wilting on the contents of free amino acids in tomato leaves (ηmoles per 1 g fw), from Rekoslavskaya *et al.* [40].

Amino acids	State of leaves		Wilted turgescent
	turgescent	wilted	
Asp	249	596	2.4
Glu	691	753	1.1
Pro	141	9167	65.0
Gly	24	53	2.2
Ala	271	152	0.6
Val	41	1222	29.8
Cys	<5	22	>4.4
Met	<5	6	>1.2
ILeu	27	379	14.0
Leu	23	275	12.0
Tyr	34	923	27.1
Phe	11	1439	130.8
Trp	<5	313	>62.6
His	<5	167	>33.4
Lys	<5	60	>12.0
Arg	<1	395	>395.0
Total	**<1533**	**15,922**	**>10.4**

The increase of free amino acid content (including Trp) without their malonylation was observed during other stresses besides drought [1, 2, 39].

Total non-protein Trp content was greater than free Trp content because MT appeared also during wilting of tomato leaves [40]. The increase of amino acid content during water deficit was communicated also in other publications [39-42]. The increase of Trp content during leaf wilting may be the result of activation of proteolysis or its synthesis during drought stress.

2.2. The Induction of Malonyltryptophan Formation with Water Deficit and Other Stresses

Plants are exposed to different stresses during their life. The question to be answered is, whether the induction of MT accumulation is a specific reaction to water stress or it can occur during other kinds of stress also. In the work of Markova *et al.* [43] excised tomato leaves were exposed to different stress factors: 1) water stress up to a loss of 30% of the initial fresh mass by drying; 2) incubation in water solutions of mannitol and polyethylene glycol (PEG1500); 3) salt stress by incubation in 1% NaCl water solution; 4) temperature stress by incubation at 4˚C and 41˚C. It may be seen in **Table 2** that floating excised leaves in solutions of mannitol 0.5 M and PEG150015% induced approximately the same increase of Trp and MT contents as the leaf wilting did. Floating in 1% NaCl solution increased only Trp content, whereas MT did not appeared. The increase of Trp content caused with NaCl was more significant than that induced by drying or incubation on mannitol and PEG solutions. However, the increases of Trp + MT content were just of the same magnitude (1002, 1018 and 1319 in mannitol, PEG1500 and NaCl solutions, and 1218 at wilting). So, more significant Trp content increase induced by NaCl can be explained by the absence of its use for the MT synthesis in this case. When the wilted leaves maintained 2 days at 4˚C the activation of Trp accumulation decreased and MT formation did not take place. Both Trp and MT accumulations were weakened at 41˚C.

Thus, only water deficit induced MT formation. Salt, cooling and heating per se did not influence its formation.

The osmotics (mannitol, PEG1500) penetrate through the cell wall, but not through the plasmalemma. Thus, water is removed from the plasma, but not through the cell wall, what induced plasmolysis. Salt penetrates through the cell wall and the plasmalemma. Because of that, only wilting and osmotics of low molecular weight induced plasmolysis and the appearance of MT, but salts induce stress without plasmolysis and do not induce MT appearance. It may be concluded that the signal for the induction of MT appearance is the cell dewatering which accompanied with cell plasmolysis. Water removing from cells can

Table 2. The effects of different stresses on MT and Try content in excised tomato leaves, from [43].

Variants	Content, *η*mol/g fw	
	MT	L-Trp
Freshly excised leaves	0	<10
Floating on mannitol solution 0.5 M 3 days	683	319
Floating on PEG1500 solution 15% 3 days	604	414
Floating on 1% NaCl solution 3 days	0	1319
Wilted leaves after 2 days at:		
4˚C	0	165
20˚C	857	361
41˚C	109	286

proceeds also with hydrofiling substances of great molecules which do not penetrate through cell wall (for example, PEG with molecular mass above 6000 kD). They cause cytorrisis (tightening cells with cell their walls without plasmolysis). As a consequence, cells are pressed with the diminishing their sizes. It is unclear today, if MT appearances occur at the cytorrisis.

2.3. The Dependence of Malonyltryptophan and Tryptophan Accumulation on the Extent and Duration of Water Deficit

Trp and MT contents increased when the leaf dewatering was induced by the increasing wilting extents and durations, mannitol concentrations, but parts of Trp in the sum of MT + Trp decreased (**Table 3**) [43]. It may be assumed that the use of accumulated Trp for MT synthesis outstripped its synthesis at the increase of induced drought.

2.4. Wilting-Induced Malonyltryptophan Appearance in Plants of Different Taxa

MT appearance during leaf wilting was found at first in tomato, potato and wheat [40]. The question was arose as to how widely this phenomenon is distributed in the plant kingdom.

Plants of several species were gathered from the territory of the institute (Irkutsk) and around it. They were tested for their ability to form MT during wilting. Leaves were excised, dried up to a loss of 30% - 50% of their fresh weight and placed in a glass camera for thin-layer chromatography with wet filter paper to

Table 3. Accumulation of Trp and MT during water deficit in excised tomato leaves induced with different extents, durations and mannitol concentrations (*ηg* per 1 g initial fw) (transformed from [43]).

Water deficit			
%	MT	Trp	Trp, %[b]
10[a]	184	391	68
20	289	246	46
40	779	350	31
50	1610	140	8
Duration, days			
1[c]	147	344	70
3	335	602	64
6	665	562	46
7	1233	628	30
Mannitol, M			
0.1[a]	31	267	89
0.3	430	764	64
0.5	696	592	46
0.7	1103	328	34

[a]after 3 days; [b]% from MT + TPП; [c]at 30% of water removed.

prevent further weight loss. They were used for Trp and MT determination after 3 days [40].

As shown in **Table 4**, Trp content in turgescent leaves varied from traces to 35 - 40 to 220 - 980 *η*mol per 1 g fw in wilted leaves (increased for 25 - 80 times) independently from their ability to form MT. This Trp was destroyed by L-amino acid oxidase what indicates the absence of D-Trp appearance at wilting. It was observed that measurable amounts of MT appeared due to leaf wilting in plants of 36 species. Very low contents (<10 *η*mole/g fw) were registered in plants of 9 species and MT was not found in plants of 24 species (**Table 4**).

Thus, significant differences were revealed between plants species in their ability to form MT during leaf wilting.

There were also differences in MT accumulation induced by water deficit between species of the same genera and between varieties of the same species.

2.5. N-Malonylation and N-Acetylation of Tryptophan

AT and some unidentified (Ehrlich-positive) indole compounds were found in extracts of some plants besides MT [40, 44]. It appeared as a consequence of leaf wilting in *Anetum graveolens* (traces), *Beta vulgaris*, *Trifolium repens*, *Heracleum sibiricum.* MT appeared simultaneously with AT in *T. repens* and *H. sibiricum.* When leaves of *T. repens* were infiltrated with D-Trp before wilting, MT became the prevalent compound and AT was seen as traces (Rekoslavskaya *et al.*, 1986). The ability of plants to acetylate and malonylate exogenous D-Trp was studied by Zenk and Scherf [31]. They found that bacterial and algal species were not able to acylate D-Trp and fungi can acetylate but not malonylate them. Lycopodiinae did not transform D-Trp to AT and MT. Many (but not all) species of other taxones (Equisetae, Coniferae, Cycadinae, Ginkgoinae, Mono- and Dicotyledonae) showed the ability to malonylate exogenous D-amino acids, but AT was not found in them. However, the data of Rekoslavskaya *et al.* [40] showed that the ability to acetylate exogenous D-Trp is observed in some Dicotyledonae plants. Moreover, it was shown that AT appeared during wilting even without D-Trp addition. The presence of L-AT in *Ephedra distachia* was communicated by Song *et al.* [37] and in plants of other species by Markova and Gamburg, [30]. Yu *et al.* [13] found AT and MT in tomato young leaves; in Ginkgo male flowers only AT; in coconut milk MT and AT. It should be noted that only a few plant species have been studied in the publications mentioned. But it should be stressed that in the course of plant evolution the ability to acetylate D-amino acids appeared first and later it was substituted by the ability to malonylate them.

2.6. The Ability of Plants to Malonylate D-Tryptophan

D-amino acids present in plants so as in other living organisms [45]. Their contents are regulated by amino acid racemase in the synthesis [46] and D-amino acid oxidase in the degradation [47]/The question arises of whether plants that were incapable of forming MT during wilting (see **Table 4**) are capable malonylating exogenous D-Trp. In order to obtain an answer to this, D-Trp was infiltrated into the excised leaves of chicory, sweet pepper, Peking cabbage incapable and white clover and fern (*Matteuccia struthiopteris* (L. Tod.) capable to form MT during wilting [48]. Then, the petioles of these leaves were immersed in water for 3 days in conditions excluding wilting. The amount of D-Trp obtained by infiltration was equal to 0.4 *μ*moles/g fw. It was established that plants which were incapable of forming MT during wilting (pepper, chicory and Peking cabbage) were capable of forming it after infiltration of D-Trp in their leaves. Besides, AT also appeared in pepper, chicory, *T. repens* and fern leaves. Kutaćek and Kefely [49] showed also that cabbage and maize which did not form MT during wilting (see **Table 4**) malonylated added D-Trp. Based on the data presented and those of other authors (see review [16]) *it may be supposed that almost all higher plants have the ability to malonylate or acetylate exogenous D-Trp independent of their ability to form MT and AT during leaf wilting.*

2.7. Are Malonyl Derivatives of Other Amino Acids Besides Tryptophan Synthesized during Wilting?

It is known that plants can malonylate other exogenous D-amino acids besides of D-Trp [14, 16, 50].

Table 4. Effects of wilting on the contents of Trp and MT in excised leaves of plants of different taxones (from [40]).

Species	Trp		MT
	Before wilting	After 3 days of wilting	After 3 days of wilting
Melilotus albus Medik.[a]	-	680	1300
Medicago sativa L.	-	490	1110
Vicia faba L.	-	980	1280
Trifolium repens L.	-	370	280
Phaseolus vulgaris L.	-	510	810
Pisum sativum L.	39	530	340
Glycine max L.	-	-	1850
Solanum tuberosum L.	11	440	1660
S. melongena L.	<10	270	<10
Lycopersicon esculentum Mill.	22	550	550
Capsicum annuum L.	35	590	0
Cucumis sativus L.	<10	490	350
Citrullus lanatus (Thunb.) Matsum.	<10	400	280
Brassica oleracea var. *capitata* L. Pers.	<10	200	0
B. oleracea var. *botrytis* L. Mill.	<10	160	0
B. pekinensis (Lour.) Rupr.	10	490	0
Cichorium intybus L.	traces	270	0
Helianthus annuus L.	-	-	92
H. tuberosus L.	-	-	106
Lactuca sativa L.	<10	250	0
Zea mays L.	10	800	0
Triticum aestivum L.	14	590	300
Beta vulgaris L.	-	410	440
Heracleum sibiricum L.	-	310	530
Anetum graveolens L.	-	630	390
Daucus sativus (Hoffm.) Roehl.	-	220	110
Malus baccata (L.) Borkh. s. l.	-	390	410
Chenopodium album L.	-	410	970
Rosa rugosa Thunb.	-	360	<10
Rubus saxatilis L.	-	240	<10

[a]- not determined.

Rosa and Neish [23] supposed that *N*-malonyl-D-Phe (MPhe) is an endogenous compound in barley leaves. Ogava and Fukuda [51] found *N*-malonyl-Ala in pea seedlings. The question arises, whether malonyl derivatives of other amino acids can appear during wilting. Malonyl derivatives of Phe and Ala were not observed in wilted tomato leaves [8]. It was proved by 1) the analysis of extracts from these leaves for the presence of *N*-acylated amino acids besides of MT and 2) the use of [^{14}C]-malonate for the labeling amino acids metabolites in wilted tomato leaves. Thus, it may be supposed that only MT is formed during wilting of plant leaves, whereas malonyl derivatives of other amino acids do not appear. Probably, this is related to the unknown necessity of many, but not all plants to neutralize the excess of Trp appeared during wilting in order to be alive in drought stress conditions. But it is necessary to study plants of other species for the confirmation of this supposition.

3. STEREOCONFIGURATION OF ENDOGENOUS MALONYLTRYPTOPHAN

3.1. Previous Opinions

Most authors who found endogenous MT in the past time believed that it contains D-Trp [15, 21, 22, 24, 28, 29, 31-33, 36, 37, 40, 43, 52] and it was suggested that L-Trp was a precursor of MT only after its conversion to D-Trp. This opinion coincided with the presence of D-amino acids in plants [7, 45-47].

3.2. Endogenous Tryptophan Conjugates in Plants Contained Mainly L-Tryptophan

In time, publications appeared in which *N*-acyl derivatives of endogenous L-Trp were found. Liang and Anderson [44] communicated that *N*-acetyl-L-Trp was contained in cultural liquid of fungus *Claviceps purpurea.* Then, Song *et al.* [37] found MT in tissue culture of *Ephedra distachya* and showed that it contained L-Trp. This contradicted to widespread in that time opinion that endogenous MT contains D-Trp. Marumo and Hattori [52] ascertained at first that immature pea seeds contained 4-Cl-D-Trp and the product of its malonylation. But later, the same group of authors [53] defined more precisely that pea seeds contained 4-Cl-L-Trp and its malonyl derivative. Markova and Gamburg [30] showed that endogenous MT isolated from cell cultures, seeds and seedlings and wilted leaves of several plants contained L-Trp. However, traces of MT containing D-Trp were found also in cell culture of ginkgo and wheat and rye seedlings. Liu *et al.* [38] proved also that MT isolated from tomato leaves contained L-Trp. MT and AT isolated recently from young tomato leaves, ginkgo male flower (pollen cone), coconut milk [13] and MT isolated from fruits of the South-American plant *Pithecellobium dulce* (Roxb.) Benth. [36] were shown to be the derivatives of L-Trp. *It may be concluded that endogenous MT contains L-Trp in most cases.*

4. THE ROLE OF N-MALONYLTRANSFERASES IN MALONYLTRYPTOPHAN FORMATION

The malonylation of several substances is catalyzed by *O*-malonyl- and *N*-malonyltransferases with the use malonyl-CoA as a donor of malonyl residue [54] Intensive study of *N*-malonyltransferases began after the discovery of malonylated ACC in plants [26, 27]. Kionka and Amrein [55] observed *N*-malonyltransferase activity in homogenates of mung bean hypocotyls and found that it malonylated not only ACC, but also D-amino acids, including D-Trp. They isolated three isoforms of *N*-malonyltransferase which malonylated D-Trp, anthranylic acid and 3,4-dichloroanilin. The first of them malonylated also ACC, but not L-Trp. Su *et al.* [56] isolated *N*-malonyltransferase from mung bean seedlings which malonylated ACC, D-Phe and D-Met using fractionating with different concentrations of ammonium sulphate. Almost all activity has been found in hypocotyls. They proposed that all substrates were malonylated with the same enzyme. Liu *et al.* [57] showed with the use of stereoisomers of 1-amino-2-ethylcyclopropanecarboxylic acid that *N*-malonyltransferase catalyzed the malonylation of only D-isomer. Guo *et al.* [58, 59] isolated *N*-malonyltransferase with molecular mass 55-kDa which was able to malonilate ACC and D-Phe. D-Trp and D-Tyr inhibited concurrently the malonylation of ACC and D-Phe. It was supposed that they were malonylated with the same enzyme. Benichou *et al.* [60] isolated another *N*-malonyltransferase with 36 kDa molecular mass from etiolated mung bean seedlings. It was different from 55 kDa *N*-malonyltransferase by

pH and temperature optimums, but was also inhibited with D-Phe. However, D-Trp and D-Tyr did not exert concurrent inhibition. This *N*-malonyltransferase was capable of using succinyl-CoA instead of malonyl-CoA, but did not use acetyl-CoA.

Unlike the preceding authors, Liu *et al.* [38] and Wu *et al.* [61] found *N*-malonyltransferase in wilted tomato leaves which was able to malonylate both D-Trp and L-Trp. ACC and Trp were not concurrent inhibitors. Consequently, ACC-malonyltransferase and Trp-malonyltransferase may be different enzymes. Crude enzyme preparation was separated into three zones of 49, 46 and 43 kDa with SDS-PAGE. Martin and Saftner [62] isolated different *N*-malonyltransferase from tomato fruits and seeds. It had molecular mass 38 kDa, was a monomer and was not inhibited with D- and L-amino acids.

The characteristics of *N*-malonyltransferases studied by all authors mentioned are summarized in Table 5. It may be seen that most of them malonylated ACC and D-amino acids. It was postulated that the same enzyme is responsible for malonylation of ACC and D-amino acids [55-59]. Guo *et al.* [59] determined that *Km* of *N*-malonyltransferase was 48 mM for D-Phe as a substrate, whereas it was equal to 500 mM for ACC. This showed that D-Phe was malonylated more effectively than ACC. The ability of *N*-malonyltransferase to malonylate L-Trp was observed only in the work of Wu *et al.* [61].

It was shown above that some plants can produce *N*-acetyl derivatives of exogenous D-amino acids in addition to MT. It was established that exogenous and endogenous L-Trp can also be transformed to AT [13, 30, 43]. Evidently, acetyl-CoA may be used for acetylation of D-amino acids and L-Trp and it may be supposed that specific *N*-acetyltransferases exist for their acetylation. However, it was shown that only malonyl-CoA and succinyl-CoA were used for derivatization of ACC, whereas acetyl-CoA and butyryl-CoA were inactive [55, 60, 62].

It may be supposed that some plants possess specific *N*-malonyltransferase which can malonylate only L-Trp. The properties of this hypothetical *N*-malonyltransferase may be: 1) the ability to malonylate only L-Trp, but not D-Trp, other D-amino acids and ACC, 2) its absence in turgescent leaves of plants capable of synthesizing MT during wilting and in turgescent and wilted leaves of plants which not able to do that. These peculiarities may be used in future for the isolation of the gene coding its amino acid sequence. The specific N-acetyltransferase which putatively appeared also during AT synthesis needs for searching and study in future.

Table 5. Characteristics of *N*-malonyltransferases isolated by several authors.

Authors	Objects	kDa	Substrates			
			ACC	D-amino acids	D-Trp	L-Trp
[54]	Soybean cells	48		Anilin derivatives		
[55]	Mung bean hypocotyls		+[a]	+	+	[c]
[56]	- " -		+	+	+	[b]–
[57]	- " -		+	+		
[58, 59]	- " -	55	+	+	+	
[60]	- " -	36	+	+	–	
[61]	Tomato leaves	48	–	+	+	+
[62]	Tomato fruits and seeds	38	+	–	–	
[63]	Peanut seedlings		+		+	

[a]ability to malonylate, [b]no ability to malonylate, [c]empty cells—absent of data.

5. POSSIBLE FUNCTIONS OF MALONYLTRYPTOPHAN IN PLANTS

Good and Andreae [21] had no ideas on the role of MT in plant physiology. The physiological significance of MT appearance during wilting remained unknown also in the modern time. It may be supposed that accumulated MT can release Trp after the restoration of the normal water status and it may be used for some syntheses.

5.1. The Influence of Dewatering on the Contents of Tryptophan and Malonyltryptophan

Good and Andreae [21] observed no decrease of MT content per one tomato plant after 11 days from their treatment with Trp. Rekoslavskaya *et al.* [48] observed that accumulation of MT and Trp induced by drought stress was hindered, but did not stop when excised and wilted tomato leaves regained their turgor as a result of dewatering. Jiao *et al.* [64] communicated that the contents of free amino acids (particularly proline) and abscisic acid is lowered to the initial level on recovery from water deficit. But the content of malonyl-ACC (MACC) was not changed. The conversion of ACC to MACC was thought to be physiologically irreversible, with the resulting product MACC being an inactive end product [26]. Rekoslavskaya *et al.* [48] observed that intact tomato seedlings resumed growth after eliminating their wilted state. This was accompanied with the diminishing MT content per fresh weight basis, but its quantity calculated for the entire plant did not change (unpublished). *All this allows to suppose that malonylation of endogenous ACC and Trp seems to be irreversible.* On the contrary, exogenously applied MT and MACC can release Trp and ACC [65, 66].

5.2. Malonyltryptophan as a Possible IAA Precursor

Because Trp is a precursor of IAA in plants [9], it was supposed that MT may be used also for IAA synthesis after its break-out with Trp release [66]. The appearance of IAA after treatment of plants with MT may be determined by its auxin-like activity. Elliott [28] did not observe stimulation of oat coleoptiles' sections elongation (standard biotest for auxin-like activity) due to their treatment with MT. He concluded that MT did not possess the auxin-like activity. But Ludwig-Muller and Hilgenberg [65] showed that treatment of Chinese cabbage with MT led to its conversion to IAA, indolylacetonitryl and indolylacetaldoxime. Enzyme preparations exerted this conversion only from MT containing L-Try.

Rekoslavskaya and Gamburg [66] and Rekoslavskaya [67] used MT preparations obtained from soybean cells cultured with D-Trp for the estimation of their auxin activity. It was shown that, instead of stimulating, MT inhibited oat coleoptile section growth. These data are in accordance with the data of Elliott [28]. It is possible that the short time duration of this biotest (20 - 24 hours) was insufficient for the release of a biologically active IAA concentration from MT.

Auxins are necessary for the growth of plant cell and tissue cultures [68]. Their influence is estimated usually after several days of cultivation. Rekoslavskaya and Gamburg [66] used an auxin-dependent soybean cell culture was used for the estimation of the influence of 1-naphthaleneacetic acid (NAA), IAA, MT and D-Trp on its growth after 21 days of cultivation [66, 67]. It was shown that the growth of cell biomass did not occur, if auxin (NAA) was excluded from the medium. NAA, IAA, MT or D-Trp included into the medium caused growth stimulation proportionally to their concentrations. It was observed that MT 30 μM caused the same growth stimulation as IAA 1 μM. D-Trp was less active than MT because its significant part was converted to MT.

No endogenous IAA was found in soybean cells cultured without NAA. It appeared when NAA or MT was included into the medium. Incubation of soybean cells with MT containing [^{14}C]-D,L-Trp resulted in the appearance of labeled Trp and IAA [40]. All that is in accordance with the assumption that exogenous MT can release Trp which can be transformed to IAA. *However, there are no evidences that endogenous MT can be the source of endogenous IAA.*

L-Trp is the precursors of IAA [12] and melatonin (AMT) [4] which can affect positively on the resistance to drought stress. But the conversion of some part of additional Trp which appeared during water deficit to MT can diminish unnecessary IAA and AMT syntheses which may be inhibitory at the super

optimal concentrations. Perhaps this is the reason for plants to use L-Trp malonylation as an additional way for the regulation of indole compound contents during drought stress.

6. DISCUSSION

MT formation during leaf wilting, first observed by Rekoslavskaya and Gamburg [32] and confirmed by other authors [38, 61] is one of many metabolic plant reactions to water deficit. It was induced not only by leaf wilting but also by mannitol and PEG. Due to this fact, a significant part of Trp, the content of which sharply increased during water deficit, becomes metabolically inert. The malonylation of other amino acids does not proceed in spite of the fact that their content increased also due to the leaf wilting. ACC is the only exception. Its transformation to MACC is stimulated by water deficit [69]. Other stresses (salt, low and high temperature) did not induce MT formation if they are not accompanied with water deficit [43].

MT formation takes place in plants of many species. However, some plants are unable to react to water deficit by MT formation. But all the plants studied were able to malonylate exogenous D-Trp even without water deficit. It was determined that water deficit mainly induced malonylation of endogenous L-Trp during wilting [30, 38]. *N*-malonyltransferases exist in plants which could bind malonate from malonyl-CoA to ACC and D-amino acids. *N*-malonyltransferase which appears during water deficit and malonylated only L-Trp has been not found yet.

It is not known today why it is necessary for plants to convert a significant part of their L-Trp into MT during leaf wilting. A significant contribution to this ambiguity is caused by the fact that not all plants have this necessity, but they do not suffer from this. The malonylation eliminates L-Trp from participation in active growth processes which may be not desirable during wilting. Malonylation of ACC is also used by plants for the regulation of free ACC content and ethylene production at drought stress. Possibly, malonylation of L-Trp and ACC permits the prevention of excessive synthesis of phytohormones – IAA, ethylene and AMT. There are some publications on the positive influence of Trp on the drought stress [70-74]. It is possible that this effect is related to the use of Trp for IAA and AMT syntheses which increased drought resistance [4, 5, 75].

63 years have passed since the first communication of Good and Andreae [21] on the presence of MT in plants. However, the question as to the necessity of its formation for plants remains unanswered up to now. If the search for the gene responsible for L-Trp malonylation during wilting will be successful, the possibility for the genetic engineering of plants capable or incapable of forming MT will appear. The study of the physiological and biochemical behavior of these transformants will help to understand the significance of MT formation for plant life during water deficit.

7. CONCLUSION

1) The content of Trp and other amino acids is increased significantly during leaf wilting in all plants studied.

2) Drought stress induces the synthesis of MT in plants of many species, but some numbers of them do not react to it by this way. A sub-group of plants can be distinguished which forms only trace amounts of MT. AT also appear in some plants.

3) Almost all higher plants can malonylate exogenous D-Trp and other D-amino acids in turgescent and wilted leaves, but MT which appeared during water deficit is synthesized from L-Trp.

4) Leaf wilting does not cause *N*-acylation of other L-amino acids besides Trp.

5) Exogenous MT can release Trp which transformed further to IAA, but no data found that this may occur from endogenous MT appeared during leaf wilting.

6) *N*-malonyltransferases are found in plants which able to transform ACC into MACC. Some of them can malonylate D-Trp and other D-amino acids also. But *N*-malonyltransferases which appeared during water deficit and can malonylate L-Trp were not observed to this time.

7) *How malonyltryptophan accumulation during drought stress can influence on animals?* Trp is a necessary component of nourishment of humans and animals, but they obtained it only from some dietary

sources such as plants, fungi, bacteria and animals. Green leaves are an important component of food and feed and the main source of Trp and other amino acids (mainly free non-protein one) [76]. It is shown (see above) that drought stress increased substantially their contents in green leaves. There are no problems in that for all amino acids besides Trp, which undergoes to malonylation leading to the accumulation of significant amounts of MT (see **Table 4**). However, it is unknown how it can influence on animals when they eat wilted leaves and if MT may be destroyed in animals with the release of Trp. Some suspicion that MT may be not indifferent for animals health occur from the publication of Lopez *et al.* [36] who found that MT is highly toxic for *Hymenolepis nana* (intestinal parasite of humans and especially of children). So, the investigation of MT metabolism in animals and its influence on them may be an important target for future studies.

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CONFLICTS OF INTEREST

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