Effect of Maleic Hydrazide Foliar Application on Biochemical Parameters and Phytohormonal Variations in Groundnut (cv. Dh 86)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

This study assessed the impact of varying concentrations of maleic hydrazide (MH) on seed biochemical parameters in groundnut. Where increasing MH concentrations resulted with least total dehydrogenase activity (0.505 OD value at A_{480}) and electrical conductivity (200 µS cm^{-1}), and increased phenol content (3.48 mg/g) at foliar spray of MH @ 3000 ppm over the control (0.822 OD value at A_{480}, 257 µS cm^{-1}, 2.80 mg/g) respectively. Further this study examines the impact of MH foliar spray at 3000 ppm, unsprayed control and a dormant check (Dh 8) on groundnut seed

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phytohormones, comparing results to an unsprayed control. Abscisic acid (ABA) levels increased from 15.6 ng/g in unsprayed control to 45.96 ng/g in MH treated seeds and 74.58 ng/g in Dh 8, promoting dormancy. Gibberellins (GA_3) reduced by 98.7% in MH treated seeds (0.88 ng/g) and by 99.2% in Dh 8 (0.54 ng/g). Auxins (IAA) decreased 86.8% in MH treated seeds (1.15 ng/g) and 22.9% in Dh 8 (6.70 ng/g) relative to unsprayed control (8.71 ng/g). These shifts highlight the distinctive effects of MH and dormant cultivar Dh 8 on dormancy regulation compared to control.

1. INRODUCTION

Groundnut (*Arachis hypogaea L.*) is a significant crop globally, valued for its both oilseed and food uses. It is known as the "king of oilseed crops" and it is believed to have been initially domesticated and cultivated in the Paraguay Valley. It was introduced to India during the first half of the sixteenth century.

Groundnut is an important oil seed crop in India, with a significant area under cultivation and production. In world India ranks first in terms of groundnut acreage, with an area of 49.61 lakh ha, and second in production, with 102.97 lakh tonnes with productivity of 2.075 kg/ha. The major groundnut growing states in India are Gujarat, Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Rajasthan, Maharashtra, and Karnataka, which contribute to 90 per cent of the total groundnut area in the country (Anon, 2023).

Understanding the biochemical basis of seed dormancy is essential, as it is largely governed by hormonal regulation that involves a precise balance between inhibitory and stimulatory compounds within the seed (Khan, 1977). Gibberellins (GAs) are key promoters of germination, while hormones like cytokinins and ethylene also play significant roles in managing dormancy and germination (Whitehead & Nelson, 1992). Moreover, phenolic compounds contribute to dormancy by inhibiting germination through their impact on cell elongation (Bewley & Black, 1982). Enzymatic activity, which tends to be lower in dormant seeds and higher in non-dormant seeds, further influences the progression of germination (Barros et al., 2014).

Ethylene acts as an antagonist to ABA during seed development, influencing dormancy induction, while cytokinins, which promote cell division, may play a role in this process during embryogenesis. Cytokinins are also known to break dormancy in seeds of various species, including apple and peanut (Cohn & Butera, 1982). Brassinosteroids (BRs) participate in multiple developmental functions, such as promoting cell elongation, cell division, and supporting pollen development and fertility. Given that a hormone's physiological effects are determined by its endogenous concentrations, precise quantification of endogenous hormone levels is essential. Phytohormone quantification has been carried out in several crops, including lettuce, oat, pea, Arabidopsis, and bitter gourd, for studies on dormancy and germination (Tang et al., 2011).

Groundnut is a important rain-fed oilseed crop in India, but its yield is significantly lower compared to the global average. Enhancement of groundnut pods is very important. A major challenge in groundnut cultivation is *in situ* germination, which arises due to a lack of seed dormancy, with some varieties exhibiting lower levels of abscisic acid. In the view of above facts this research investigation was carried out to assess the effect of maleic hydrazide foliar application on biochemical parameters and phytohormonal variations in groundnut.

2. MATERIALS AND METHODS

During *kharif* 2023 the field experiment was conducted in the All India Coordinated Research Project on Groundnut, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka. The highly sensitive genotype Dh 86 with respect to *in situ* germination was selected for this experiment. Experiment was conducted in randomized complete block design with 11 treatments and three replications. i.e. T_0: Un sprayed control, T_1: Foliar spray of maleic hydrazide @ 500 ppm, T_2: Foliar spray of maleic hydrazide @ 1000 ppm, T_3: Foliar spray of maleic hydrazide @ 1500 ppm, T_4: Foliar spray of maleic hydrazide @ 2000 ppm, T_5: Foliar spray of maleic hydrazide @ 2500 ppm, T_6: Foliar spray of maleic hydrazide @ 3000 ppm, T_7: Foliar spray of maleic hydrazide @ 3500 ppm, T_8: Foliar spray of maleic hydrazide @ 4000 ppm, T_9: Foliar spray of maleic hydrazide @ 4500 ppm and T_{10}:

Foliar spray of maleic hydrazide @ 5000 ppm. The spacing between plants and rows were 0.10 m and 0.30m, respectively. Field experiment was carried out in 2023 *Kharif* season in the All India Coordinated Research Project on Groundnut, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka.

In order to prepare a solution of 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm, 3000 ppm, 3500 ppm, 4000 ppm, 4500 ppm and 5000 ppm concentrations, 0.5 g, 1.0 g, 1.5 g, 2.0 g, 2.5 g, 3.0g, 3.5 g, 4.0 g, 4.5 g and 5.0 g of the Maleic hydrazide chemical powder was dissolved in 1 litre of distilled water respectively. Then mixture was solubilized by adding KOH pellets with the use of magnetic stirrer. Maleic hydrazide was sprayed at 70 days after sowing except control plot (T_0). After attaining maturity, the plants were left in the field for 15 days with regular watering for the germination of pods *in situ.* After 15 days, the pods were lifted, and the number of pods sprouted in each treatment was counted.

The total phenolic content (TPC) of groundnut extracts and seeds were determined by spectrophotometric method with Folin-Ciocalteu's reagent, (Arun et al., 2016). The electrical conductivity (EC) of groundnut seeds leachate was determined by as per the procedure developed by anonymous (2015) and expressed in µS cm^{-1} and total dehydrogenase activity (A_{480}) of the seeds was determined by method described by Perl et al. (1978). Selected one *in situ* germination tolerant treatment (Foliar spray of MH @ 3000 ppm), and un sprayed control (Dh 86) was taken along with one dormant check (Dh 8) were subjected for Profiling of phytohormones by LCMS method (Pan et al., 2008). The sample analysis made through outsource (IIHR, Bengaluru).

3. RESULTS AND DISCUSSION

Total dehydrogenase activity (TDH), an important indicator of seed viability, decreased significantly with increasing maleic hydrazide concentrations. The highest total dehydrogenase activity (0.822) was recorded in the control (T_0), while the lowest (0.505) occurred at foliar spray of maleic hydrazide at 3000 ppm (T_6). Decreased TDH activity reflects impaired metabolic processes within the seed, particularly those related to energy production and cell division, both of which are crucial during germination. Seed dormancy

and germination are intricately regulated by various biochemical factors. Research has shown that naturally occurring inhibitors play a crucial role in preventing seed germination by targeting specific enzymatic activities (Elliott and Leopold, 1953). One key indicator of cellular vitality in seeds is dehydrogenase, a respiratory enzyme whose presence and activity signify living cells (Jain et al., 2013). The relationship between enzyme activity and dormancy is further evidenced by studies showing significantly reduced enzymatic activity in dormant seeds (Barros et al., 2014) and dehydrogenase Activity positively associated with the seed vigour and field emergence (Jyotirmaye et al., 2022).

Electrical Conductivity (EC), which measures seed membrane integrity, decreased with rising concentrations of maleic hydrazide. The lowest electrical conductivity (200 µS cm^{-1}) was observed in the foliar spray of maleic hydrazide at 3000 ppm (T_6), compared to 257 µS cm^{-1} in the un sprayed control. A decrease in electrical conductivity suggests decreased membrane permeability. Electrical conductivity is closely linked to ionic concentration. Increased solute leakage, reflected by higher electrical conductivity in seed leachate, indicates greater membrane permeability. This is often associated with reduced seed vigour, poor field emergence, and diminished storability in many crops (Presley, 1958). These findings are in line with results obtained by Jyotirmoyee et al (2022) in groundnut.

Phenol content in seeds exhibited an increasing trend with higher maleic hydrazide concentrations, reaching a peak at 3.48 mg/g in the foliar spray of maleic hydrazide at 3000 ppm (T_6) as compared to control (2.80 ng/gm). Application of plant growth retardants (PGRs) can modify plant metabolism, either suppressing or enhancing the production of plant secondary metabolites (Lemanowicz et al., 2023). Phenolic compounds are known for their role in plant defense mechanisms (Kumar, 2022) and their increase in response to maleic hydrazide application likely indicates a stress response. Application of maleic hydrazide leads to upregulation of key genes related to and biosynthesis of secondary metabolites (Phukan et al. 2016). Saini (2024) reviewed that phenolic compounds accumulate in plants under abiotic stress, acting as antioxidants to mitigate damage from reactive oxygen species (ROS).

Table 1. Effect of maleic hydrazide foliar application on seed biochemical parameters in groundnut (cv. Dh 86)

Treatments	IG (%)	TDH Activity (OD value at A_{480})	EC ($\mu S\ cm^{-1}$)	Phenol (mg/g)
T$_0$: Un sprayed control	55.99 (48.42)	0.822	257	2.80
T$_1$: Foliar spray of maleic hydrazide @ 500 ppm	36.01 (36.86)	0.763	255	2.84
T$_2$: Foliar spray of maleic hydrazide @ 1000 ppm	32.30 (34.62)	0.681	247	2.87
T$_3$: Foliar spray of maleic hydrazide @ 1500 ppm	29.67 (32.99)	0.633	246	2.91
T$_4$: Foliar spray of maleic hydrazide @ 2000 ppm	29.41(32.82)	0.604	243	3.12
T$_5$: Foliar spray of maleic hydrazide @ 2500 ppm	26.92 (31.24)	0.584	224	3.31
T$_6$: Foliar spray of maleic hydrazide @ 3000 ppm	24.64 (29.75)	0.505	200	3.48
T$_7$: Foliar spray of maleic hydrazide @ 3500 ppm	25.02 (30.00)	0.510	221	3.46
T$_8$: Foliar spray of maleic hydrazide @ 4000 ppm	25.60 (30.38)	0.520	227	3.44
T$_9$: Foliar spray of maleic hydrazide @ 4500 ppm	25.10 (30.07)	0.526	223	3.45
T$_{10}$: Foliar spray of maleic hydrazide @ 5000 ppm	25.65 (30.47)	0.518	220	3.43
Mean	30.57	0.61	233	3.19
S.E.m ±	0.941	0.012	4.034	0.066
CD @ 1%	2.777	0.049	16.081	0.261
CV	15.99	3.501	2.995	3.557

Figures in the parentheses are arcsine transformed values.

TDH : Total dehydrogenase   EC : Electrical Conductivity
IG : In situ germination

Table 2. Effect of foliar spray of maleic hydrazide on phytohormonal variation in groundnut

Treatments	ng/g of tissue (mean)	Percent change over control	Fold change	SD	t-stat.	SEM	P (T<=t) Two tail
Abscisic Acid (ABA)							
T_0	15.60			0.25		0.147	
T_1	45.96	194.30	2.94	1.98	26.4	1.149	0.00005*
T_2	74.58	378.40	4.78	0.11	373.8	0.065	0.00001*
Gibberellic Acid 7 (GA$_7$)							
T_0	0.11			0.007		0.004	
T_1	0.10	-13.8	0.86	0.007	2.81	0.004	0.05*
T_2	0.12	+6.00	1.06	0.02	0.63	0.01	0.57
Gibberellic Acid 4 (GA$_4$)							
T_0	121.08			1.92		1.11	
T_1	79.77	-34.10	0.66	2.68	-21.70	1.55	0.0005*
T_2	45.23	-62.70	0.37	0.47	-66.60	0.27	0.0005*
Gibberellic Acid 3 (GA$_3$)							
T_0	70.52			1.08		0.62	
T_1	0.88	-98.7	0.01	0.005	-111.96	0.003	0.00*
T_2	0.54	-99.2	0.008	0.04	-112.05	0.020	0.00*
3-Indole Acetic Acid (IAA)							
T_0	8.71			0.05		0.03	
T_1	1.15	-86.8	0.13	0.10	-105.9	0.06	0.00*
T_2	6.70	-22.9	0.77	0.20	-16.9	0.12	0.00*
3-Indole Butyric Acid (IBA)							
T_0	1.05			0.09		0.06	
T_1	0.78	-26.1	0.74	0.05	-4.53	0.03	0.015*
T_2	0.89	-15.8	0.84	0.04	-2.81	0.02	0.049*
Benzyl aminopurine (BA)							
T_0	2.20			0.09		0.06	
T_1	1.46	-33.8	0.66	0.09	-9.48	0.06	0.0001*
T_2	0.60	-72.6	0.28	0.05	-26.20	0.03	0.0001*
Zeatin trans isomer (ZTI)							
T_0	1.17			0.04		0.02	
T_1	0.75	-36.0	0.64	0.04	-13.56	0.02	0.00*
T_2	0.74	-36.9	0.63	0.04	-13.94	0.02	0.00*

Contd...

Treatments	ng/g of tissue (mean)	Percent change over control	Fold change	SD	t-stat.	SEM	P(T<=t) Two tail
Trans zeatin Riboside (TZR)							
T_0	27.00			0.69		0.39	
T_1	26.15	-3.20	0.96	0.75	-1.45	0.43	0.21
T_2	15.71	-41.9	0.58	0.12	-28.04	0.07	0.00*
Salicylic acid (SA)							
T_0	14523.56			239.23		138.41	
T_1	13509.49	-6.99	0.93	256.99	-13.54	148.68	0.0001*
T_2	11887.45	-18.12	0.82	144.69	-16.34	83.57	0.0001*
Jasmonic Acid (JA)							
T_0	0.20			0.01		0.007	
T_1	0.23	13.6	1.14	0.04	1.22	0.02	0.28
T_2	0.22	8.40	1.08	0.01	1.81	0.006	0.12
Cis-Jasmone (CJ)							
T_0	189.13			1.64		0.95	
T_1	1139.66	502.10	6.02	2.95	487.34	1.71	0.00*
T_2	698.91	269.4	3.69	4.17	197.77	2.41	0.00*
Methyl Jasmonate (MJ)							
T_0	1.09			0.043		0.03	
T_1	0.79	-27.4	0.73	0.02	-10.71	0.012	0.00001*
T_2	1.68	53.8	1.54	0.03	18.44	0.02	0.00*
1-AminoCyclopropane-1-Carboxylicacid (ACC)							
T_0	2.94			0.05		0.03	
T_1	1.37	-53.40	0.47	0.04	-39.29	0.02	0.00*
T_2	1.46	-50.50	0.49	0.05	-37.13	0.02	0.00*
Epibrasinolide (EPIBRA)							
T_0	34.00			1.79		1.03	
T_1	15.92	-53.30	0.47	0.94	-15.50	0.54	0.00013*
T_2	27.67	-18.7	0.81	1.84	-4.28	1.06	0.0132*

In each test group [T_0: Unsprayed control, T_1: Foliar spray of maleic hydrazide @ 3000 ppm and T_2: Dh 8 (Dormant Check)] in each replicate and such replicates were subjected to an independent variable 'T' test analysis. *Represents the significant (P ≤ 0.05) difference between the control and treatment.

Phytohormones present in the seed play a crucial role in the induction of seed dormancy. We analyzed different classes of phytohormones such as auxins, gibberellins, abscisic acid, cytokinins, and defense hormones (salicylic acid, jasmonic acid, and cis-jasmonate) to understand their levels and contribution in promoting dormancy and inhibiting germination. This was done by selecting the best treatment of a foliar spray of maleic hydrazide (MH) at 3000 ppm (T_1) and comparing it to Dh 8 (Dormant Check, T_2) and the control (T_0).

The plant hormone abscisic acid (ABA) plays an important role in regulating seed dormancy and germination. ABA biosynthesis is required for the induction of primary dormancy during seed development and in general it is an inhibitor of germination (Bewley et al., 2013). Unsprayed (control) showed lower level of ABA (15.60 ng/gm) but in foliar spray of maleic hydrazide (MH) at 3000 ppm and in the dormant check Dh 8 ABA level (45.96 ng/gm & 74.58 ng/gm) increased by 2.94 and 4.78 folds respectively. Interestingly experimental results in the present study shows decreased germination percent in foliar spray of maleic hydrazide (MH) at 3000 ppm and in dormant check Dh 8. This was mainly due to the increased ABA content. Similar findings were found that the expression of some key genes of ABA signaling pathway and ABA response was up-regulated after MH treatment in *S. polyrrhiza* (Zhu et al., 2021).

Gibberellic acids (GA) are plant growth hormones and have positive effects on seed dormancy release and germination, stem elongation, flowering initiation, and flower and fruit development. Among gibberellic acid class of hormones, GA_3 showed decreasing trend *i.e.*, it was decreased by 0.01 folds in foliar spray of MH at 3000 ppm (0.88 ng/gm), whereas in dormant cultivar Dh 8 (0.54 ng/gm) showed drastic changes in GA_3 content and it was decreased by 0.008 folds compared to control (70.52 ng/gm). The biosynthesis of bioactive gibberellins (GAs) occurs in two main phases. Early steps are catalyzed by enzymes like ent-copalyl diphosphate synthase (CPS), ent-kaurene synthase (KS), ent-kaurene oxidase (KO), and ent-kaurenoic acid oxidase (KAO). These enzymes convert precursor molecules into intermediates in the GA pathway. The later steps involve enzymes such as GA_2 oxidase (GA_2ox), GA_{20} oxidase (GA_{20}ox), and GA_3 oxidase (GA_3ox), which belong to the 2-oxoglutarate-dependent Fe (II) oxygenase superfamily. These

enzymes are encoded by different gene families. Their activity is regulated by developmental and environmental cues. The later-stage enzymes play crucial but opposing roles in the regulation of bioactive GA levels, affecting plant growth and development (Hedden and Phillips, 2000). upregulation of GA_{20}ox and GA_3ox increases the GA level, whereas higher expression of GA_2ox decreases the GA level (Schomburg et al. 2003; Lo et al. 2008). Whereas Two homologs of Arabidopsis GA_2ox enzymes were upregulated in response to MH-treatment in our Axillary bud dataset, which lowers the concentration of GA and inhibits Axillary bud development (Singh et al., 2020).

The key role of auxin is a signaling molecule that coordinates seed life. Auxin is involved in the development of the endosperm, seed coat and essential compound involved in integuments development. Recent biochemical and genetical evidence supports the involvement of auxins in Physiological dormancy of seeds and complement the role of ABA (Shashi et al., 2003) in seed germination. However, in groundnut seed it was found that 3- indole acetic acid (IAA) content decreased by 0.13 and 0.77 folds in foliar spray of MH at 3000 ppm (1.15 ng/gm) and dormant cultivar Dh 8 (6.70 ng/gm) as compared to control (8.71 ng/gm). The Indole-3-Butyric Acid (IBA) levels followed a similar pattern. The similar trend was observed in pea seeds were MH acts as an anti-auxin by inhibiting cell elongation and division, which are primary effects of auxin activity. Auxins, such as indole-3-acetic acid (IAA), promote cell elongation, root growth, and apical dominance in plants. MH counteracts these processes by interfering with auxin-regulated growth, thus inhibiting plant development (Leopold and Klein, 1952). MH acts as an anti-auxin or a regulator of auxin metabolism (Hoffman and Parups, 1964).

Cytokinins are the major plant hormones that regulate numerous aspects of plant growth and development, such as cell division, apical dominance, root formation, stomatal behaviour, and chloroplast development. But in seeds also they are involved in promotion of germination and release of seed dormancy by acting antagonistically to ABA action. Among the different cytokinin studied benzyl aminopurine and zeatin trans isomer recorded significant increase in their levels in both the treatments. Level of benzyl aminopurine was significantly (0.66 and 0.28 folds) lower in foliar spray of MH at 3000 ppm (1.46 ng/g) and dormant cultivar Dh

8 (0.60 ng/g) as compared to control (2.20 ng/g). The similar trend was observed in zeatin trans isomer but with respect to trans zeatin riboside dormant cultivar Dh 8 showed non significant as compared to control. As the level of cytokinin increased it promoted seed germination, it means cytokinin content increased from dry seeds to germinating seeds (Bicalho et al., 2015, Wang et al., 2019) in palm seeds. The foliar application of maleic hydrazide may have caused an increase in ABA levels and a decrease in GA content, leading to variations in cytokinin accumulation. The similar findings were found in tobacco that Application of maleic hydrazide inhibits bud growth by suppressing the expression of key Transcription factors in meristem development and by lowering cytokinin biosynthesis through altering expression of STM-like KNOX transcription factors (Singh et al., 2020).

Defense hormones such as salicylic acid (SA), jasmonic acid (JA), cis-jasmonate and methyl jasmonate showed differential response among the treatments. Salicylic acid levels were lower in foliar spray of maleic hydrazide (MH) at 3000 ppm (13509.49 ng/g) and dormant cultivar Dh 8 (11887.45 ng/g) as compared to control (14523.56 ng/g) and these treatments were decreased by 0.93 and 0.82 folds respectively as compared to control. From the observation we can conclude positive relation between salicylic acid and gibberellic acid which decreased after MH treatment. Because SA levels were low in MH treated and in dormant check Dh 8. Interestingly Jasmonic acid (JA) levels were increased in both the treatments by 1.14 and 1.08 folds compared to control. In addition, Previous studies in Arabidopsis suggest that WRKY50 and WRKY51 act as positive regulators of salicylic acid (SA) mediated signaling and negative regulators of JA signaling with respect to seed germination (Gao et al. 2011). But these signaling pathways are altered by MH treatment in Tobacco which decreases the SA content and increases the JA content (Singh et al.,2020). JA is having negative role in induction of germination and some reports supported that JA inhibit hypocotyl elongation and seedling growth in Arabidopsis (Chen et al., 2013).

Cis- jasmone which is a defensive plant hormone it increased significantly in both the treatments. There is no much information available regarding role of cis- jasmone in regulating seed germination and dormancy. However, some findings suggested that its level will be higher when plants get damaged by insect pest (Birkett et al., 2000). So, under stress condition cis jasmone level will be increased. Methyl jasmonate levels also decreased significantly in foliar spray of MH at 3000 ppm (0.79 ng/g) and increased in the dormant cultivar Dh 8 seeds (1.68 ng/g) compared to control (1.09 ng/g). This observation suggests that methyl jasmonate having negative relation with dormancy release and germination so methyl jasmonate content was decreased in MH Sprayed treatment compared to control and these observations are supported by Staswick et al. (1992) in Arabidopsis wherein inhibition of primary root growth was observed.

In Epibrasinolide and 1-aminocyclopropane-1-carboxylic acid (ACC) showed significant relation with control, foliar spray of MH at 3000 ppm and Dh 8 (Dormant Check) and we couldn't find any role regarding these two hormones in this study.

4. CONCLUSION

The study demonstrates that foliar application of maleic hydrazide (MH) at 3000 ppm significantly impacts the biochemical and phytohormonal profiles of groundnut seeds (cv. Dh 86), promoting seed dormancy. Increased phenol content and reduced total dehydrogenase activity and electrical conductivity highlight the physiological changes induced by MH. Additionally, MH treatment elevated abscisic acid (ABA) levels while significantly reducing gibberellin (GA_3) and indole-3-acetic acid (IAA) levels, aligning with enhanced dormancy regulation. The dormant check (Dh 8) exhibited a similar hormonal trend, with even higher ABA and lower GA_3 levels compared to MH treatment, indicating its inherent dormancy-promoting attributes. These findings underscore the efficacy of MH as a dormancy-inducing agent and provide valuable insights into its potential application in managing seed dormancy and germination in groundnut.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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