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Effect of Moisture and Salt Stress on Oil Filling in *Brassica* Seeds

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Oil filling was adversely affected by moisture and salt stress in *Toria* and *Raya*. Among various moisture stress conditions, the stress throughout the growth and stress before flowering were more detrimental than stress after flowering to affect lipid deposition in the *Toria* seed. Higher salt stress affected oil accumulation more severely than moderate salt stress. Salt and moisture stress increased erucic acid content at the cost of oleic and linoleic acids in *Brassica* seeds.

Key Words: Moisture stress, Salt stress, Oil filling, *Brassica* seeds

Introduction

Moisture and salt stress are known to affect many aspects of plant growth by modifying their anatomy, morphology, physiology and biochemistry (Kramer 1969, Poljakoff-Mayber & Gale 1975). A previous study carried out in this laboratory (Sukhija et al. 1976) has shown that restricted irrigations drastically affected oil synthesis in the developing seeds of *Rocket Salad (Eruca sativa* Mill). Similar effects have also been observed in sunflower (Gupta 1982) where salt and moisture stress reduced oil filling by affecting the rate of photosynthesis in the leaves, transport of metabolites from the leaves to the seeds and synthesis of fatty acids at the peak period of oil filling. The present study aims at ascertaining as to whether moisture stress at various stages of growth affects oil filling in *Toria*. In addition, another aspect of research relating to the effects of various salt stress conditions on the developing *Raya* seed has also been covered.

Materials and Methods

The seeds of *Toria* (*Brassica campestris* var. *Toria*) were sown on 30 September, 1984 and those of *Raya (Brassica juncea* L.) on 28 November, 1984 in pots lined with polythene bags. Each pot contained 6 kg of loamy soil (*p*H 8.2 and electrical conductivity (EC) 0.5 mmhos/cm). Each pot was supplied with recommended basal doses of nitrogen as urea, phosphorus in the from of KH_2PO_4 and sulphur in the form of K_2SO_4.

For moisture stress studies, four levels were kept: (i) Control—100% field capacity, (ii) Stress before flowering (SBF)—60% field capacity up to flowering followed by 100% field capacity up to maturity, (iii) Stress after flowering (SAF)—100% field capacity up to flowering followed by 60% field capacity up to maturity, and (iv) Stress throughout growth (STG)—60% field capacity. Soil maintained at 60 and 100% field capacity had 4.4 and 10 g water/100 g soil, respectively, in the available range, i.e., 0.3–15 bar suction of moisture availability. Irrigation was given on alternate days and loss of water due to transpiration and evaporation was compensated by weighing the pots.

For salt stress studies, three salinity levels were kept: (i) control, (ii) S_1 (EC—4.0 mmhos/cm), and (iii) S_2 (EC—8.0 mmhos/cm). The desired salinity level was created by adding a mixture of salts of NaCl, Na_2SO_4 and $CaCl_2$ in the ratio of 2 : 1 : 0.5.

The developing seeds were collected at 20, 30 and 40 days after fertilization (DAF) and at maturity. The extraction of lipids was done by the method of Folch et al. (1957) and the estimation of various lipid classes, fatty acid make-up and (1-^{14}C) acetate incorporation as reported earlier (Sukhija et al. 1984b).

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Results and Discussion

Physiological Para neters and Yield

The plants followed normal growth pattern in the 'control' but the plants under moisture stress were weak and had a tendency to bend. Flowering was delayed by 8–10 days in STG plants. Plant height was maximum in the 'control' and reduced equally in SBF and SAF. Moisture stress considerably decreased seed and straw yield (table 1) and the maximum decrease was recorded in STG followed by SBF and SAF treatments. During initial stages of salt stress condition there was a notable reduction in plant height, number of leaves and flowering branches per plant. However, during later stages, there was hardly much difference in S_1 and S_2 conditions. There was a decrease in seed yield without any appreciable effect on straw yield of the stressed plants (table 1).

Dry Matter and Oil Accumulation

There was a consistent and parallel increase in the dry matter and oil content, both in *Toria* (table 2) and *Raya* (table 3). At initial stages, the oil content was low and reached its peak between 20 and 40 DAF in *Toria* and 20 and 30 DAF in *Raya*. Apart from the species difference between the two crops, the observed difference may also be due to other factors including the environment. These results are in confirmation of the results reported by Norton and Harris (1975) and Sukhija et al. (1984a).

In *Toria*, moisture stress affected dry matter (mg/100 seeds) accumulation as well as the oil (mg/100 seeds) deposition. The effect was more pronounced in STG in comparison to other treatments. Similar adverse effect of restricted irrigations on oil accumulation in *taramira* (Sukhija et al. 1976) had been observed. Osman and Talha (1975) have also indicated that irrigations to provide 100% field capacity (FC) in comparison to 40, 60 and 80% FC were useful for optimum oil synthesis in sunflower. In *Raya*, the pattern of dry matter accumulation showed a consistent increase between 20 and 40 DAF after which there was negligible change. Under salt stress conditions, S_2 (higher stress) adversely affected the oil accumulation. Such a low content of oil could be attributed to low photosynthetic rate (Gale 1975) and reduced translocation of photosynthates from leaves to seeds (Pakroo & Kashired 1981).

Table 1 *Seed and straw yield (g/pot) in* Toria *and* Raya *as influenced by moisture and salt stress, respectively*

Toria			*Raya*		
Treatment	Seed	Straw	Treatment	Seed	Straw
Control	34.5	25.0	Control	22.9	15.3
SBF	28.1	21.0	S_1	16.2	13.1
SAF	29.4	20.8	S_2	14.0	10.5
STG	25.3	18.5			

Each figure is an average of three replications

Table 2 *Incorporation of (1-^{14}C) acetate into total lipids of developing* Toria *and* Raya *seeds as influenced by moisture and salt stress (nmoles/g fresh weight)*

Treatment	*(Toria)*			Treatment	*(Raya)*	
DAF	20	30	40		20	30
Control	4.4	5.1	0.23	Control	4.2	4.1
SBF	3.8	5.7	0.22	S_1	1.8	2.0
SAF	3.8	5.9	0.29	S_2	2.8	2.1
STG	3.9	6.3	0.24			

Each figure is an average of three determinations

Lipid Composition

The lipid composition in the control set was similar to our previous results (Gupta 1982). The higher amounts of phospho- and glyco-lipids in moisture stress indicates that the plant probably synthesizes membrane lipids in response to the adverse effects of water stress. Similar responses have been observed in sunflower (Chetal et al. 1980). In salt stress, the effect was opposite to that of moisture stress with respect to phospho- and glyco-lipids. A reduction in triglycerides and an increase in free fatty acids with salt stress might be due to degradation of triglycerides; the effect was not observed in case of moisture stress. The content of sterol was decreased significantly with salt stress only and this may be related to the lower availability of precursors for their synthesis. In *Raya*, the amount of phospholipids was higher at 20 DAF and decreased thereafter. The relative amount of glycolipids, free fatty acids and triglycerides remained almost constant in *Raya* whereas in *Toria*, phospholipids increased and other lipid components remained more or less constant. The total sterols, a minor component of the lipids, interestingly, registered increase in the seed during maturity. Moisture and salt stress had negligible effect on lipid composition (tables 3, 4).

Acetate Incorporation

The rate of fatty acid synthesis determind by (1 ^{14}C) acetate incorporation into total lipids was low at 20 DAF, increased at 30 DAF and again decreased at 40 DAF in *Toria* (table 2). The moisture stress had negligible effect on acetate incorporation. On the contrary, the rate of fatty acid synthesis was the same

Table 3 *Oil content and relative percentage of various lipid classes of developing* Toria (Brassica campestris) *seeds as influenced by moisture stress*

Treatments	Control				SBF				SAF				STG			
DAF	20	30	40	M	20	30	40	M	20	30	40	M	20	30	40	M
Seed weight (mg/100 seeds)	88	149	238	279	146	188	249	293	75	148	229	342	93	165	220	278
Dry matter (%)	20	38	63	75	24	44	59	80	19	32	61	68	21	29	57	58
Oil content (mg/100 seeds)	21	56	116	139	16	69	106	136	12	46	83	175	14	46	95	135
Oil content (%)	24	38	49	50	11	36	43	46	16	31	36	51	16	28	45	19
Phospholipids (%)	0.02	0.07	0.14	0.13	0.05	0.13	0.20	0.14	0.04	0.17	0.16	0.16	0.06	0.23	0.18	0.16
Glycolipids (%)	0.96	0.84	1.00	0.84	1.02	0.84	0.96	0.70	1.02	0.96	1.40	1.00	1.00	1.02	1.22	1.02
Free fatty acids (%)	0.28	0.34	0.40	0.40	0.17	0.28	0.27	0.34	0.19	0.14	0.14	0.14	0.14	0.17	0.34	0.27
Triacylglycerols (%)	96	96	94	95	97	96	96	95	98	97	97	97	97	97	95	96
Sterols (%)	0.09	0.11	0.32	0.47	0.05	0.04	0.25	0.30	0.06	0.08	0.40	0.58	0.08	0.09	0.37	0.32

Critical difference ($P<0.05$)	DM %	Oil %	Phospholipids	Glycolipids	FFA	TG	S
Moisture stress (M)	7.0	3.0	0.02	0.21	0.08	2.5	0.07
Developing stages (D)	10.0	4.1	0.83	0.08	0.07	2.8	0.06
Interaction (M × D)	5.9	2.7	0.02	0.07	0.07	2.4	0.06

Table 4 *Oil content and relative percentage of various lipid classes in developing* Raya (Brassica juncea *L.*) *seeds as influenced by salt stress*

Treatments	Control				S_1				S_2			
DAF	20	30	40	M	20	30	40	M	20	30	40	M
Seed weight (mg/100 seeds)	140	262	394	401	102	268	403	409	101	250	411	419
Dry matter (%)	20.9	36.3	47.5	61.5	17.2	30.8	45.4	74.3	16.1	32.1	45.2	59.9
Oil content (mg/100 seeds)	16	95	142	175	12	97	149	159	15	82	124	130
Oil content (%)	11.3	36.3	36.1	43.6	11.5	36.2	37.0	38.9	14.7	32.3	30.2	31.1
Phospholipids (%)	0.2	0.14	0.13	0.05	0.17	0.2	0.06	0.02	0.16	0.13	0.02	0.02
Glycolipids (%)	0.96	0.84	0.70	0.22	1.02	1.22	0.96	0.7	1.4	1.0	0.7	0.42
Free fatty acids (%)	0.14	0.17	0.19	0.28	0.17	0.19	0.28	0.34	0.19	0.17	0.19	0.40
Triacylglycerols (%)	97.03	97.2	96.8	96.5	97.1	96.6	96.1	95.5	96.0	97.0	97.0	95.0
Sterols (%)	0.09	0.11	0.4	0.47	0.05	0.08	0.09	0.32	0.06	0.25	0.32	0.25

Critical difference ($P>0.05$)	DM %	Oil %	Phospholipids	Glycolipids	FFA	TG	S
Salt stress (S)	7.0	3.2	0.02	0.19	0.09	2.4	0.09
Developing stages (D)	9.2	4.1	0.07	0.07	0.06	2.3	0.07
Interaction (S × D)	8.1	3.7	0.05	0.07	0.07	2.3	0.08

at 20 and 30 DAF in case of *Raya* and a decreased rate of lipid synthesis was observed as compared to control. This may be due to the reduced availability of precursors (Gale 1975).

Fatty Acid Composition

Both *Toria* and *Raya* seed oils had palmitic, oleic, linoleic, linolenic and erucic acids as the main fatty acid components. There were many small chain fatty acids and their amounts pooled together have been expressed as small chain fatty acids (tables 5 and 6). With the maturity of the seed, there was an increase in the amount of erucic acid with a concomitant decrease in the amount of oleic acid. The amount of 16 : 0 fatty acid did not differ appreciably under moisture stress conditions. However, a decrease in the proportion of erucic acid under these conditions was noted. It is known that erucic acid is synthesized by sequential

Table 5 *Fatty acid composition (% weight basis) of developing* Toria *seed as influenced by moisture stress*

Treatments	Control				SBF				SAF				STG			
DAF	20	30	40	M	20	30	40	M	20	30	40	M	20	30	40	M
Fatty acids																
Small chain	17.6	1.8	2.0	0.8	3.7	5.4	2.6	2.9	1.5	2.0	2.0	2.5	17.6	1.0	2.0	1.8
14:0	1.2	1.7	2.7	Tr	5.1	0.5	3.2	Tr	3.3	0.4	2.5	Tr	1.2	1.7	1.0	Tr
16:0	6.1	4.1	4.5	3.0	6.0	7.6	8.4	5.7	14.2	5.2	3.5	3.5	6.1	7.1	10.5	17.0
18:1	32.6	12.0	15.3	19.3	35.2	33.1	22.6	22.5	22.0	17.5	20.2	20.0	37.6	16.0	15.0	19.0
18:2	21.2	16.0	14.1	15.1	32.1	30.7	12.4	10.0	23.6	13.2	15.2	19.6	21.2	16.0	14.1	16.0
18:3	4.9	6.0	7.1	9.1	11.1	5.0	15.2	11.5	20.5	18.7	14.4	11.8	4.9	13.0	11.4	9.0
20:1	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
22:1	16.0	58.4	54.3	52.7	12.2	17.7	35.6	47.4	14.9	49.0	42.2	42.6	11.4	44.4	45.0	46.0

Table 6 *Fatty acid composition (% weight basis) of developing* Raya *seed as influenced by salt stress*

Treatments	Control				S_1				S_2			
DAF	20	30	40	M	20	30	40	M	20	30	40	M
Fatty acids												
Small chain	2.3	4.9	2.9	0.9	2.3	2.6	2.2	1.5	7.0	2.0	2.9	3.3
14:0	2.2	2.4	1.1	Tr	1.9	1.5	2.2	Tr	1.0	2.3	1.7	Tr
16:0	12.2	6.9	3.7	4.0	10.6	6.8	4.3	4.3	11.7	9.7	3.1	1.0
18:1	25.5	19.8	13.8	15.4	31.8	24.7	13.3	18.2	50.2	20.1	12.3	22.1
18:2	22.9	11.8	8.0	13.0	24.1	16.8	9.2	16.4	16.5	16.0	14.7	12.7
18:3	5.2	22.2	9.8	7.8	8.8	9.3	8.6	10.2	4.7	17.2	17.9	18.2
20:1	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
22:1	30.9	32.0	60.7	58.9	28.2	38.2	60.3	49.4	8.9	32.8	39.2	42.8

two-step elongation of oleic acid (Norton & Harris 1975) and the decrease might be attributed to the inhibition of the chain elongation process. The conversion of oleic acid to erucic acid was affected after 40 DAF (Sukhija & Bhatia 1972). A pronounced decrease after 40 DAF in SBF indicates that moisture stress plays an important role in erucic acid biosynthesis. Whether the inhibition of the conversion of oleic to erucic acid is caused by lesser availability of ATP and reducing potential in the cell under moisture stress or some inhibitors of the elongation process is yet an open question. Under salt stress conditions, the proportion of erucic acid decreased at 20 DAF; the decrease being more in S_2. In mature seeds also, the content of erucic acid decreased appreciably under higher salt stress conditions. Such a decrease in erucic acid with a corresponding increase in oleic and linoleic acids might be attributed to salt accumulation in the cell which inhibits the conversion of oleic acid to erucic acid as pointed out above in the case of moisture stress. Thus, it is suggested that the biosynthesis of erucic acid is a very sensitive process which is influenced in different ways under different physiological stresses and requires further systematic investigations.

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