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Amino Acids Associated with Femaleness of Castor (*Ricinus communis* L.) Induced by Kinetin and Morphactin

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Kinetin and morphactin increased the number of female flowers. An increased female tendency is associated with increase in amino acids quantitatively. Male flowers are characterized by a higher concentration of proline, tyrosine, histidine, hydroxyproline and serine. Female flowers are characterized by a higher concentration of aspartate, glutamate, threonine and alanine. A possible downward transport of proline, lysine, tyrosine and histidine and an upward transport of other amino acids is visualized. Uniform occurrence of proline in all parts of plant at the culmination of reproductive phase appears to be a diagnostic feature of femaleness caused by kinetin and morphactin.

Key Words: **Amino acids, Castor, Femaleness, Kinetin, Morphactin**

Introduction

Modification of sex expression by kinetin and morphactin has been reported by several workers. Induction of feminization has been reported by treatment with cytokinins (Catarino 1964), benzyladenine in *Vitis vinifera* (Negi & Olmo 1966). Morphactins significantly affect sex expression in some cucurbits such as *Cucumis sativus* (Robinson et al. 1971), *Cucumis melo* (Bisaria 1974), *Cannabis sativa* (Mohan Ram & Jaiswal 1971), *Nicotiana paniculata* (Sankhla 1969) and *Carica papaya* (Jindal & Singh 1976). Physiological studies associated with sex expression are scanty. Studies on chlorophyll pigments, carbohydrates (Choudhuri et al. 1957) and amino acid composition (Kasinathan et al. 1965) of *Carica papaya* provided no physiological marker of sex identification in the vegetative seedlings. The present study has been designed to associate amino acids with flower sex expression both in the vegetative and reproductive phases.

Materials and Methods

Castor (*Ricinus communis* L. var. Aruna), a radiation induced mutant developed from HC-6 was selected for this study. The plants were raised in plots at Sri Venkateswara University Botanical Garden, Tirupati. They flowered eleven weeks after sowing. Kinetin (6-furfurylaminopurine) and morphactin

EMD 7301 W (methyl ester of chlorfluerenol) were used at a concentration of 20 ppm each, as they were found to be more effective in increasing femaleness. The aqueous solutions of the chemicals with 0.01% wetting agent (Tween 20) were sprayed to the plants at 6 and 8 weeks after sowing, till the point of run-off, while the controls were sprayed with distilled water. The shoot tips, flowers, leaves, stems and roots were collected soon after the second spray and at one-week intervals thereafter. The Second stage was conspicuous by the critical vegetative phase, and the third by the critical reproductive stage where the shoot tip possessed minute female and male flowers. At the fourth stage female and male flowers formed were collected and their sex expression was determined.

Free amino acids were estimated in various organs as under : 5 g of freshly harvested material was refluxed on a water-bath with 80% ethanol for half an hour for complete extraction of alcohol-soluble compounds. The boiled material was ground in a mortar using additional volume of 80% ethanol and centrifuged. The supernatant was collected and the alcohol was removed *in vacuo*. The water-soluble fraction thus obtained was passed through Dowex 50-X8 (H^+ ; 20-50 mesh) column, and the amino acids were eluted with 50 ml of 2N NH_4OH. The eluate was then treated to dryness in vacuo and the residue was dissolved in 1 ml of 95% alcohol. Amino acids were separated on chromatograms using secondary butanol : formic acid : water (75 : 13 : 12, v/v) and buffer (6.3 g sodium citrate + 3.7 g potassium dihydrogen orthophosphate in 100 ml water) saturated phenol for first and second directions respectively. The developed and air-dried chromatograms were sprayed with 0.2% ninhydrin in *n*-butanol. The identification of the individual amino acids was made by the comparison of Rf values with that of standards, which were run simultaneously. The coloured areas were cut and the amino acid content was estimated according to the method of Giri et al. (1952).

Results and Discussion

Sex expression

The results showed that the ratio of pistillate to staminate flowers in control plants was 1 : 4.75. There was an increase of 78.13% over control with kinetin and 41.63% with morphactin treatment in femaleness. With morphactin treatment, the reduction in maleness was only 3% but the increase in femaleness was 40% and hence the ratio of pistillate to staminate was not significantly altered. Kinetin reduced male flowers and increased the production of female flowers to greater degrees and hence the ratio is significantly altered. The critical difference (C.D.) values showed significant difference between control and treatments with respect to female flowers. But the production of male flowers with morphactin treatment is insignificant with respect to control. Increase in femaleness with kinetin treatment is in correlation with the reports of earlier workers in different plants (Negi & Olmo 1966, Catarino 1964). Increase in femaleness with morphactin is in correlation with the

Table 1 *Effect of kinetin and morphactin on changes in sex expression* (Mean of ten replication)

	Control	Kinetin	Morphactin
Female flowers	32	57	45
Male flowers	152	92	148
Ratio of females : Male	1:4.75	1:1.61	1:3.29

	Female flowers	Male flowers
F calculated	23.11*	31.14*
C.D. at 5% level	7.53	17.56

*Significant at P=0.05

production of pistillate flowers in *Luffa acutangula* (Bisaria 1977). Sankhla (1969) reported strong supression of maleness and Krishnamoorthy (1971) reported an increase in femaleness with morphactin treatment. In the present study morphactin showed no significant effect on the maleness.

Amino acids

All the amino acids with a few exceptions tended to increase from the first stage to the third stage but decreased at the fourth stage, irrespective of the treatments. Shoot tips and leaves contained more amino acids than stems and roots. Male flowers were rich in amino acids than female flowers. Tyrosine was an extra amino acid found in roots which was absent in stems and leaves. Male flowers are characterized by a higher content of proline than female flowers. Probably proline is translocated downwards from shoot tip to the root. Amino acids lysine and tyrosine also appeared from the second stage only. Lysine appeared both in the shoot tip and the leaf at the second stage and in the root only at the fourth stage, probably indicating downward translocation when flowers were formed. Tyrosine appeared only in the shoot tip from the second stage. Tyrosine, just as proline, was also high in the male flowers. At the fourth stage tyrosine was found in the root and its absence in leaf and stem may indicate its translocation downwards. Histidine also appeared only at the third stage both in shoot tip and leaf and at the fourth stage it appeared in stems and roots also, indicating a probable downward transport. Thus, proline, lysine, tyrosine and histidine appear to exhibit a downward transport. In the amino acid composition, the male flowers were characterized by a higher concentration of proline, tyrosine and histidine predominently during the transition from vegetative to reproductive stage apparently indicates that they may act as flower-promoting substances or in someway connected with the onset of reproductive phase, particularly of maleness. This assumption can be substantiated by the observation of Tanaka and Takimato (1977) regarding the flower-promoting effect of some amino acids and amides in *Lemna paucicostata*. Nitsan (1962) in his approach to the discovery of relations between the qualitative composition of amino acids and proteins and flowering process deduced that it has not resulted in any definite conclusions. Dasgupta and Kasinathan (1966) have investigated free amino acids of male and female flowers from the cucurbitaceous plants. They showed that in *Citrullus colocynthis, Benincasa cerifera* and *Cucurbita pepo*, the female flowers contained more aspargine, aspartic acid, glutamic acid and arginine. They found further that of the 19 amino acids no single one could be characteristic of male flowers. The present study with castor could provide amino acids proline, tyrosine and histidine as physiological markers of male and aspartic, glutamic, threonine and alanine of female sexes. Hydroxyproline was exclusively found in male flowers. Serine content was also very high in male flowers. The amide glutamine was high in male flowers, while the amide asparagine was high in female flowers. A steady increase during the reproductive stage more particularly in aspartate, glutamate and serine was noticed. Aspartate, glutamate and serine are products of CO_2 fixation in photosynthesis. Apparently, greater femaleness is a sequel to high photosynthetic efficiency caused by kinetin and morphactin.

In general, with a few exceptions all the amino acids were relatively higher in the plants treated with kinetin morphactin than control. Cystine content was more in the shoot tip and leaf compared to stem and root. Kinetin caused a decrease in shoot tip and leaf and an increase in stem and root. The behaviour of morphactin was more effective in this respect. From the second stage,

Table 2 *Changes in free amino acid composition of shoot tips of control (C) Kinetin (K) and morphactin (M) treated plants (Mean of three replications)*

μg/g dry weight

Amino acids	Stage I			Stage II			Stage III		
	C	K	M	C	K	M	C	K	M
Cystine	516	384	448	327	160	79	419	298	145
Asparagine	340	349	420	340	486	461	506	531	753
Glutamine	170	189	164	235	255	292	456	414	693
Aspartic Acid	727	766	981	1173	1049	1343	2081	1951	2377
Glutamtic acid	455	533	510	692	800	820	1231	1294	1560
Serine	693	738	760	865	990	1011	1144	1248	1446
Glycine	122	105	85	56	83	62	94	152	150
Threonine	149	150	210	167	219	169	375	384	386
β—alanine	115	118	79	154	184	169	219	227	258
α—alanine	129	108	187	216	249	270	544	303	339
γ—aminobutyrate	189	119	170	241	160	174	575	243	226
Phenylalanine	88	155	210	148	261	326	619	430	597
Leu-isoleucine	54	48	74	105	101	140	200	162	252
Lysine	—	—	—	62	30	45	88	35	65
Histidine	—	—	—	—	—	—	156	177	403
Tyrosine	—	—	—	31	47	50	125	142	161
Proline	—	—	—	93	130	157	625	606	726
Arginine	—	—	—	—	—	—	165	193	207

Table 2A *Effect of kinetin and morphactin on changes in free amino acid composition of female and male flowers (Mean of three replications)*

μg/g dry weight

Amino acids	Control		Kinetin		Morphactin	
	Female	Male	Female	Male	Female	Male
Cystine	327	635	915	460	610	494
Asparagine	591	870	1235	1284	878	1274
Glutamine	303	225	460	484	278	362
Aspartic acid	1776	1565	2780	2750	1838	1753
Glutamic acid	865	735	1305	1425	1118	1067
Serine	777	1175	1635	1778	763	1476
Glycine	108	250	165	270	134	288
Threonine	352	285	535	468	283	385
β—alanine	108	210	185	328	120	226
α—alanine	318	315	495	463	360	329
γ—aminobutyric acid	295	325	445	624	312	503
Phenylalanine	523	325	445	515	307	456
Leu-isoleucine	108	310	270	468	154	343
Lysine	39	110	125	156	65	94
Histidine	254	485	175	452	120	470
Tyrosine	103	235	175	302	120	235
Proline	304	1645	615	2402	418	1857
Arginine	371	422	483	597	402	465
Hydroxyproline	—	372	—	221	—	308

Table 3 *Changes in free amino acid composition of leaves of control (C), kinetin (K), and morphactin (M) treated plants. (Mean of three replications)*

μg/g dry weight

Amino acids	Stage 1			Stage 2			Stage 3			Stage 4		
	C	K	M	C	K	M	C	K	M	C	K	M
Cystine	305	171	187	287	238	97	312	344	147	315	413	311
Asparagine	111	154	147	129	159	182	187	294	265	121	151	220
Glutamine	41	73	71	52	72	81	89	132	104	40	46	30
Aspartic acid	388	495	628	582	786	682	746	1080	938	640	927	734
Glutamic acid	833	1059	1072	892	1020	1085	900	1019	985	705	616	826
Serine	235	295	322	258	287	329	339	456	546	272	247	452
Glycine	37	60	46	59	87	74	89	128	111	71	101	75
Threonine	103	154	88	133	140	136	171	284	117	220	164	233
β—alanine	25	38	29	52	68	50	82	132	87	102	101	154
α—alanine	272	273	285	295	253	240	204	280	144	161	216	121
γ—aminobutyrate	169	90	126	173	147	120	204	223	161	232	259	292
Phenylalanine	124	175	209	166	223	240	197	243	224	285	275	331
Leu-isoleucine	34	38	67	44	64	58	59	84	67	87	144	144
Lysine	—	—	—	18	15	19	39	30	37	49	49	49
Histidine	—	—	—	—	—	—	69	125	101	108	115	92
Proline	—	—	—	20	32	41	85	105	134	80	167	266

Table 4 *Changes in free amino acid composition of stems of control (C), kinetin (K), and morphactin (M) treated plants (Mean of three replications)*

μg/g dry weight

Amino acids	Stage 1			Stage 2			Stage 3			Stage 4		
	C	K	M	C	K	M	C	K	M	C	K	M
Cystine	102	202	198	123	208	225	161	222	231	96	71	138
Asparagine	128	143	165	138	178	148	130	146	134	102	119	113
Glutamine	111	194	265	161	222	225	105	122	116	96	107	69
Aspartic acid	153	270	289	146	274	334	285	362	590	402	494	781
Glutamic acid	145	253	289	184	289	260	161	280	353	236	191	269
Serine	179	244	298	177	185	260	315	350	414	332	387	538
Glycine	51	59	66	46	44	56	37	35	49	38	48	44
Threonine	43	67	91	46	59	86	37	76	109	115	143	125
β—alanine	43	51	33	15	37	49	37	47	67	70	77	106
α—alanine	77	110	132	100	119	183	124	152	176	96	83	131
γ—aminobutyrate	128	253	165	61	128	169	192	198	316	192	202	250
Phenylalanine	34	42	74	38	37	42	49	140	164	96	149	125
Leu-isoleucine	34	42	74	38	37	42	31	41	73	32	42	69
Lysine	—	—	—	—	—	—	19	29	24	19	24	19
Histidine	—	—	—	—	—	—	—	—	—	32	30	38
Proline	—	—	—	—	—	—	—	—	—	198	191	219

Table 5 *Changes in free amino acid composition of the roots of control (C), kinetin (K) and morphactin (M) treated plants (Mean of three replications)*

μg/g dry weight

Amino acids	Stage 1			Stage 2			Stage 3			Stage 4		
	C	K	M	C	K	M	C	K	M	C	K	M
Cystine	44	21	99	88	95	126	116	116	152	81	88	132
Asparagine	61	71	54	44	66	54	90	100	95	49	48	70
Glutamine	28	36	44	20	14	9	47	42	70	18	31	31
Aspartic acid	72	96	143	69	128	63	125	167	202	121	119	162
Glutamic acid	83	107	128	64	109	131	108	103	152	188	176	263
Serine	121	162	158	88	166	149	112	170	169	148	211	233
Glycine	28	36	35	29	28	36	39	35	41	31	22	35
Threonine	28	25	20	34	47	63	52	58	62	36	62	53
β-alanine	11	15	10	15	28	27	34	35	62	31	35	57
α-alanine	55	46	44	59	57	81	60	51	49	45	57	48
γ-aminobutyrate	94	81	64	88	128	86	125	151	156	98	119	119
Phenylalanine	39	30	35	29	47	32	52	48	82	40	22	79
Leu-isoleucine	28	20	20	39	33	14	47	32	21	27	13	44
Lysine	—	—	—	—	—	—	—	—	—	13	26	18
Histidine	—	—	—	—	—	—	—	—	—	22	31	36
Tyrosine	—	—	—	—	—	—	—	—	—	58	48	26
Proline	—	—	—	—	—	—	—	—	—	36	48	114

cystine content was more in the leaf than in the shoot tip. There appears to be a downward movement of cystine in plants (Castor in the present study) with changed sex expression (with morphactin and kinetin). Cytokinins have the capacity to direct the flow of chemicals within the plant. According to Banerjee and Laloraya (1967), this may be partly a result of the high ratio of protein nitrogen to soluble nitrogen and the consequent low concentration of amino acids could induce translocation of amino acids from regions of high concentration (from shoot to lower parts or vice versa in the present study). Although this observation substantiates the results of the present study with respect to kinetin, it is of interest to note that morphactin also behaved in a similar way.

Asparagine, glutamine, aspartic acid, glutamic acid and serine contents were enhanced by kinetin treatment and to a lesser degree by morphactin. Classically the substance asparagine was thought to be a storage and translocatory form of nitrogen, and Pfeffer (1899) conceived of this substance as streaming up to growing regions, there to combine with carbon framework, from sugar, to reform protein. Amides asparagine, and glutamine and possibly the basic amino acid arginine are frequent storage forms and move from one organ to another. Much evidence indicates that the substances able to donate nitrogen in an acceptable form at the site of protein synthesis are glutamine and glutamic acid, which, with carbohydrate, readily form protein. Nevertheless, this may not be true in all plants. Kulaeva et al. (1958) found that in pumpkin plants, the amino nitrogen is transported mainly in the form of alanine, glutamic and γ-aminobutyric acids. In the present study, glycine, threonine and β-alanine showed higher values in shoot tips and leaves and not in stems and roots with kinetin treatment. Alanine, phenylalanine and leucine also showed a similar trend. An interesting point to be noted is that all the amino acids showed a higher concentration in the stems of morphactin-treated plants. Probably morphactin forms the stem as a sink. Proline appeared from the second stage onwards and only in the shoot tip. Kinetin and morphactin caused an increase in its concentration. At the third stage it appeared in the leaf and at the fourth stage (i.e. when flowers were formed in all parts including stems and roots. Morel (1965) found that the effect of cytokinins which do not appear to be the result of growth induction are changes in the tissue content of free amino acids, especially proline. Morel's observation corroborates the present study with respect to proline. The translocation of amino acids is explained on the basis of concentration gradient i.e. form regions of high concentration to low concentration as a consequence of the high ratio of protein nitrogen to soluble nitrogen based on the observation of Banerjee and Laloraya (1967) and also on the unpublished data of the authors.

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References

Banerjee D and Laloraya M M (1967) Correlative studies on plant growth and metabolism. III. Metabolic changes accompanying inhibition of the longitudinal growth of stem and root by kinetin; *Pl. Physiol.* **42** 623-627

Bisaria A K 1974 Physiology of sex expression in plants, Ph.D. Thesis, Meerut University, Meerut

—— 1977 Effect of a morphactin on growth, sex expression, fruit set and yield in *Luffa acutangula*; *Can. J. Bot.* **55** 752-756

Catarino F M 1964 Some aspects of kinetin on sex expression in *Bryophyllum crenatum*; *Portug. Acta. Biol. Ser.* **A8**, 267-284

Choudhuri R S, Garg O K and Borah P C 1957 Physiological changes in relation to sex in Papaya; *Phyton* **9** 137-147

Dasgupta J and Kasinathan S 1966 Free amino acids in male and female flowers of cucurbitaceae; *Indian J. exp. Biol.* **4** 186-187

Giri K V, Radhakrishnan A N and Vaidyanathan C S 1952 Some factors influencing the quantitative determination of amino acids separated by circular paper chromatography; *Ann. Chem.* **24** 1677-1678

Jindal K K and Singh R N 1976 Modification of flowering pattern and sex expression in *Carica papaya* by morphactin, ethephon and TIBA; *Z. Pflanzenphysiol.* **78** 403-410

Kasinathan S, Ramakrishnan S and Srinivasan B 1965 Amino acids of male and female flowers of papaya; *Curr. Sci.* **34** 211

Krishnamoorthy H N 1971 Effect of morphactin on growth and sex expression of *Luffa acutangula*; *Z Pflanzenphysiol* **65** 88-90

Kulaeva O N, Silina, E I and Kurasanov, A L 1958 Path of ammonium-nitrogen composition in pumpkin plants; *Fizol. Rast.* **4** 520-527

Mohan Ram H Y and Jaiswal V S 1971 Feminization of male flowers of *Cannabis sativa* by a morphactin; *Naturwissenschaften* **58** 149-150

Morel G 1965 in ; *Proceedings Int. Conf. Plant Tissue Culture* pp 93-101 eds Mc Cutchan, Berkeley

Negi S S and Olmo H 1966 Sex conversion in a male *Vitis vinifera* by a kinin; *Science* **152** 1624-1625

Nitsan J 1962 Electrophoretic patterns of *Xanthium* leaf extracts as affected by physiological age of leaf, photoperiod and age of the plant; *Pl. Physiol.* **37** 291-295

Pfeffer W 1899 *Physiology of Plants*. Vol. **1** Translated by A J Ewart (Oxford: Clarendon Press) 632 pp

Robinson R W, Cantliffe D J and Shannon S 1971 Morphactin-induced parthenocarpy in the cucumber; *Science* **171**, 1251-1252

Sankhla N 1969 Effect of morphactin on flower sex expression in *Nicotiana paniculata*; *Z. Pflanzenphysiol.* **61** 350-352

Tanaka O and Takimato A 1977 Flower-promoting effect of some amino acids and amides in *Lemna paucicostata*; *Pl. Cell Physiol.* **18** 27-34