IAA/GA_3 quantitative ratio of some sunflower genotypes representing CMS-Rf system

Maria Duca, Angela Port
Moldova State University, Plant Biology Department, 60, A. Mateevici Street, Chisinau, MD 2009, Republic of Moldova, E-mail: mduca2000@yahoo.com

ABSTRACT

Quantitative ratio of IAA/GA_3 (indole-3-acetic acid/gibberellic acid) was studied during the growth and development of different sunflower genotypes that represent a CMS-Rf system. It has been shown that IAA/GA_3 ratio is variable and depends on ontogenesis stages, organs and genotype. Thus, IAA/GA_3 ratio had maximal values for male sterile line and minimal ratio for restorer line RW637Rf. The highest IAA/GA_3 ratio was registered in cotyledon leaves and subsequently decreased during ontogenesis whereby the hormonal ratio in reproductive stages was higher in inflorescences than in leaves. Gibberellic acid exogenously applied increased the hormonal ratio in the male-fertile line. The specificity of IAA/GA_3 balance in male sterility-fertility phenotype expression and in GA-induced pollen sterility is discussed.

Key words: CMS-Rf system – gibberellic acid – IAA/GA_3 ratio – indole-3-acetic acid – male fertility – male sterility.

INTRODUCTION

Hormonal regulation of plant growth and development including interaction between different classes of hormones remains an important research topic in biology. Plant growth regulators, endogenous or exogenously applied, are involved in male reproductive development, regulating sex differentiation (Ciailahean, 1988) and male (genetic and cytoplasmic) sterility promoting (Luis and Durand, 1978; Kaul, 1988; Rastogi and Sawhney, 1990; Nakajima et al., 1991) at various species. Our previous work has shown that CMS sunflower lines contain lower amounts of gibberellins than fertile genotypes, including homozygote line with Rf genes (Duca et al., 2003). This evidence suggests an auxin and gibberellin interaction in microsporogenesis processes by their quantitative ratio.

It is known that auxin and cytokinin interaction plays a decisive role in cell division and elongation (Inoue et al., 1991), in induction of root and stem development (Jacobsen et al., 1995). Also, the gibberellin and abscisic acid interaction was shown to regulate the beginning of seed germination through gene expression regulation (Collett et al., 2000; Zentella et al., 2002). It is also known that GA induces synthesis and secretion of a number of hydrolytic enzymes in germinating seed endosperm (Muthukrishanan et al., 1984; Jacobsen et al., 1995), and GA activity can be suppressed by abscissic acid (White et al., 2000).

To reveal the functional role of IAA/GA_3 balance in male sterility-fertility phenotype expression, the hormonal quantitative ratio was studied during the growth and development of different sunflower genotypes, representing a CMS-Rf system.

MATERIALS AND METHODS

Plant material

Sunflower plants were cultivated in the experimental field of Moldova State University according to conventional technologies during four years. Two isonuclear lines MB514 and MB514CMS with mitochondrial *orfH522*, RW637Rf with nuclear homozygote restoration nuclear gene *Rf* and hybrid F_1 obtained by cross between these lines (MB 514 CMS x RW637Rf) with restored male fertility (*Rf_*) were chosen for analyses. For comparative studies, SW501CMS was additionally used. Phenocopies method was applied (Duca, 1998).

The treatment with exogenous gibberellic acid (GA_3) solution by plant spray was carried out at the development period of the inflorescence buds. At this stage, prior to the opening of the inflorescence, male meiosis occurs in disc flower anthers (Anaschenco, 1971). Non-GA_3 treated plants (control) were sprayed with distilled water. For assaying non-GA_3 treated plants (control) and GA_3 treated plants, 24 h post-treatment were used.

Chromatographic analysis

The plant material was collected at various vegetative stages that were correlated with development and microsporogenesis (Duca, 1998). Fresh plant material (about 10g) was homogenized and fixed in cold (-20°C) 80% acetone (1:30 ratio) and extracted over-night at 3-5ºC during 24h. After a series of organic extractions and purifications, the extracts were dried in vacuum at 40°C. The residue was dissolved in 0.1 ml N,O-bis(trimethylsilyl)-acetamid with the addition of 0.05 ml of trimethylclorosilan (1%) and then subjected to chromatography.

Quantitative analysis of phytohormones was performed using gas-liquid chromatographic method and indole-3-acetic acid and gibberellic acid (Sigma) as internal standards, as described previously by Cavell et al., 1967 with modifications (Duca et al., 1997).

The chromatograph FRACTOVAP 4200 equipped with a detector of flame ionization, line programs for temperature MOD 410, integrator MEGA SERIES SP 4270, rustproof column (2m x 4mm) with 5% SE-30 DMCS Cromoton W, 60/80 mesh (0.15-0.2 mm) was used for analysis with gas carrier N_2 - 25 ml/min. Air flow was maintained at 300 ml/min, while hydrogen flow was 25 ml/min. The injector temperature was + 210^{0}C, and the detector temperature was +210^{0}C. The phytohormones were determined in the following temperature regime: after injection, the temperature was maintained at 60^{0}C for 4 min, then the temperature rate increase was 12^{0}C/min until the temperature of 220^{0}C was achieved. This temperature was maintained until the end of the analysis. The phytohormones content was expressed in ng per gram of fresh weight (ng/g fwt).

Data are presented as means ± SE (standard errors) of three separate experiments (n = 6 for each experiment) and Student's *t* test ($P< 0.05$and $P<0.09$) was used to determine the statistical significance of differences between genotypes.

RESULTS AND DISCUSSION

In our previous work, it was shown that the IAA and GA_3 content in vegetative and reproductive tissues was variable and depends on ontogenesis stages, organs and genotype (Duca and Port, 2002; Duca et al., 2003). But the ascertained changes of IAA and GA_3 concentrations are insufficient for revealing their functional role in male fertility-sterility phenotype expression. For this purpose, IAA/GA_3 ratio was analyzed in ontogenesis of sunflower plants using CMS-Rf system.

The highest IAA/GA_3 ratio was found in the cotyledon leaves with maximal values higher than 9 (Table 1). But, the RW 637 Rf line, in contrast to the all studied lines, had the minimal hormonal ratio – 7.9 resulting from a higher gibberellins content only, because no obvious genotypic differences in auxin content were observed.

Table 1. IAA/GA_3 ratio in leaves of different sunflower genotypes

Genotype	Stages of plant growth and development				
	Cotyledons	First leaves	Bud developing	Active growth	Blossoming
F_1	9.5	4.5	4.4	4.3	2.5
MB 514	9.7	3.6	3.9	3.5	2.5
MB 514 CMS	9.7	5.9	6.1	4.6	2.9
RW 637 Rf	7.9	3.3	2.9	2.8	3.8
SW 501 CMS	-	6.6	7.4	5.5	3.6

The IAA/GA_3 ratio was twofold decreased during the first true leaves stage and its values subsequently decreased in ontogenesis down to the lowest values (2.5-3.6) ascertained at blossoming stage. Heterozygote hybrid F_1 with restored male fertility and self-pollinate homozygote male fertile lines showed almost similar values of IAA/GA_3 ratio, while male sterile plants showed the highest hormonal ratio (Table 1).

The IAA/GA_3 ratio in inflorescences tissues, as in leaves, showed higher values at male sterile plants, while male fertile genotypes had nearly the same values of IAA/GA_3 ratio (Table 2).

.

Table 2. IAA/GA_3 ratio in inflorescences of different sunflower genotypes

Genotype	Reproduction stages		
	Bud developing	Active growth of inflorescence	Blossoming
F_1	4.4	4.9	4.9
MB 514	4.1	4.4	5.0
MB 514 CMS	10.2	11.0	7.7
RW 637 Rf	3.3	3.9	5.1
SW 501 CMS	10.3	10.1	6.9

Significant results were observed for the RW 637 Rf line, which showed the lowest values of this ratio both in the leaves and in the inflorescences, assayed at the stages of bud development and active growth of inflorescence, when flower development and microsporogenesis occurred.

Similar features of the variable ratio were also determined for disc flowers (Table 3). Thus, during the flower development, the values of the studied index decreased in male sterile lines. But in comparison with male fertile genotypes, IAA/GA_3 ratio in sterile flowers was higher in archesporogenesis and sporogenesis phases. Meanwhile, in the following reproduction stage (carpogenesis) almost the same values of IAA/GA_3 ratio were found for all studied genotypes.

.

Table 3. IAA/GA_3 ratio in disc flowers of different sunflower genotypes

Genotype	Microsporogenesis stages		
	Arhesporogenesis	Sporogenesis	Carpogenesis
F_1	4.7	5.0	5.1
MB514	4.1	4.8	5.0
MB514 CMS	8.9	6.6	4.6
MB514 + GA_3	6.9	6.0	5.0
RW637 Rf	5.4	5.1	4.5
SW501 CMS	11.7	7.4	4.4

A special interest related to physiological and genetic aspects of this study represents the variation of the IAA/GA_3 ratio at isogenic lines under exogenous gibberellins treatment (Table 4).

Table 4. IAA/GA_3 ratio in plants treated with gibberellins

Lines		Post-treatment period, hours						
		0	24	48	72		96	
					leaves	inflorescence	leaves	inflorescence
MB 514	control	4.5	4.8	4.5	5.1	4.6	4.9	4.6
	+ GA_3		5.4	5.1	5.9	4.9	5.7	4.7
MB514 CMS	control	6.1	5.6	5.3	6.0	4.6	6.6	5.6
	+ GA_3		5.6	5.6	5.7	4.6	5.1	5.2

The GA3 exogenously applied at inflorescence bud developing stage increased the values of IAA/GA_3 ratio in leaves and inflorescences tissues of male fertile lines almost to the level of the values found for MB 514 CMS line. This increasing effect was significant after 72 and 96 hours post-treatment. The revealed variations in hormonal balance were not noticed for CMS plants (Table 4). In spite of these genotypes being considered hormone susceptible, the effect of GA-treatment on endogen IAA/GA_3 ratio was different. Similar effect of ``genotype correction`` to the normal hormonal status under exogenous phytohormone influence was also reported for several hormone metabolism mutants (Fadeeva et al., 1980).

The following analysis of IAA/GA_3 ratio at entire plant level (Fig. 1) provided the information on genetic and physiologic interactions in self-regulation of CMS-Rf system at sunflower (Fig. 1).

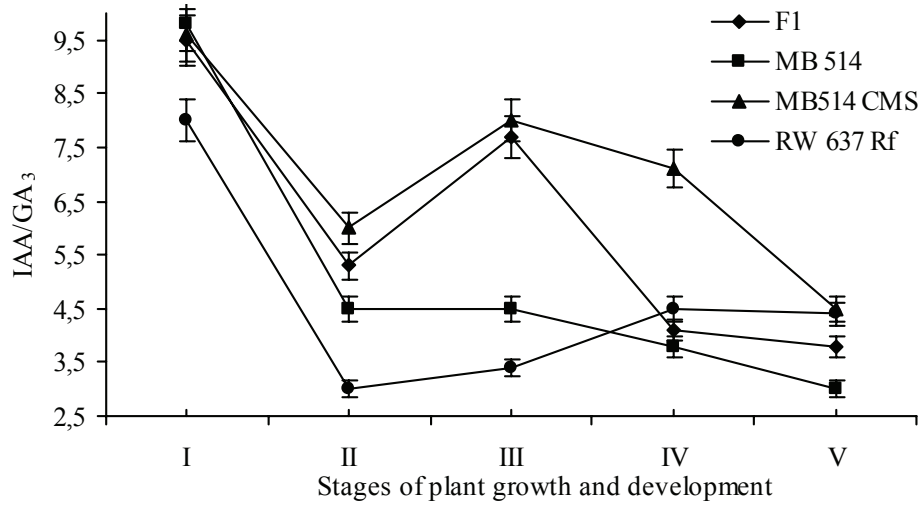

Fig. 1. Hormonal ratio of different sunflower genotypes at following stages of growth and development: I-cotyledons; II-first leaves; III- bud developing; IV- active growth; V – blossoming.

Thus, the most significant differences were revealed at the developing inflorescence bud stage. From the physiological point of view, this stage represents the stage of floral bud evocation and induction, because it was shown that microsporogenesis and microgametogenesis in sunflower occurs prior to the opening of inflorescence, when the diameter of the inflorescence bud reaches 2.5 – 4.5 cm (Smart et al., 1994). A high auxines/gibberellines ratio was ascertained at the stages of bud development (7.9) and active growth of the inflorescence (6.9) for the MB514CMS line, characterized by Srfrf genotype. A significantly lower ratio was observed for RW637 Rf (FRfRf) and MB514 (Frfrf), suggesting that the values of hormonal ratio of the CMS line are much higher than the optimal balance, which, according to our results, is approximately 4 for male fertile genotypes. The hybrid F_1 (SRf) contained sterile cytoplasm with nuclear Rf genes, which restore male fertility in homo- and heterozygote combination, resulting in the normalization of physiologic and biochemical processes in plants (Dmitreva et al., 1971). The fertility restorer gene presence in a genotype of these plants probably resulted in the IAA/GA_3 ratio decreasing at active growth and blossoming stages of reproduction development. Low values of this hormonal ratio are characteristic of fertile genotypes and high values are typical for sterile ones. The hormone ratio alterations observed at the critical stages of reproduction development, especially at microsporogenesis phases, reveal the phytohormonal mechanism of CMS-Rf genetic system control, because in F_1 the ratio of analyzed phytohormones is already restored at the next stage of the growth and development.

The hormonal balance and interactions between various plant hormones, as well as the cell capacity to receive the hormonal signal, play an important role in physiological spatial and temporal regulation of ontogenesis (Egorov et al., 1990; Braedford and Trewavas, 1994; Ross and Neill, 2001).

Our results have revealed the structural changes as a result of different auxins and gibberellins content and their ratio. Therefore, male sterile genotypes are characterized by a high IAA/GA_3 ratio. Also, the GA_3 treatment of fertile plants, resulting in phenotype male sterility, induced the increase in the IAA/GA_3 ratio, caused by the augmentation of endogen auxins and gibberellin amounts with a different intensity, which finally led to a ratio approximately similar to that in male sterile genotypes (Table 4).

It would seem that the hereditary cytoplasmic and GA-induced male sterility can be explained by the change in the phytohormone ratio and not in their concentration. It can be assumed that the phenotypic expression of the morphogenetic program, especially microsporogenesis realization, depends on the IAA/GA_3 ratio. The hormonal balance plays an essential role during the key stage of microsporogenesis (bud development and active growth of inflorescence).

These conclusions are sustained by the reported data. Thus, it was established that IAA/GA_3 regulates the primary differentiation of conductive fascicles, and, if this ratio is high, short phloem fascicles are developed (Roni et al., 1990). Also, it is well known that cytoplasmic and induced male sterility appear at the level of sporophyte tissues, because mononuclear microspores of the tetrads develop

normally up to the stage of binuclear pollen (Simonenko, 1982). This process is characterized by the break of interaction between the anther nests and parenchyma tissues of receptacle and an insufficient supply of nutritive substances (Frenchel, 1982), that finally cause tapetum tissue degeneration and the disruption of pollen formation (Roni et al., 1990).

ACKNOWLEDGEMENTS

We are grateful to Ph.D., Rotaru T. (SRC ``Magroselect``, Soroca, Republic of Moldova) for providing us with sunflower seeds and fruitful discussion during the investigation. The paper was written with support from Project Nr.4032 at Science and Technology Center in Ukraine.

REFERENCES

Anaschenco, A.V. 1971. Osobennosti virascivanii podsolnicinica pri himicescoi castrati. Selectia I semenovodsta 2:36-38.

Braedford, K.T., and A.I. Trewavas. 1994. Sensitivity thresholds in plant hormone action. Plant Physiol. 105:1029-1036.

Cavell, B.D., J.M. Millan, R.J. Pryce, and A.S. Sheppard. 1967. Plant Hormones Thin layer and gas-liquid chromatography of the gibberellins: direct identification of the gibberellins in a crude plant extract by gas/liquid chromatography. Phytochem. 6:867-874.

Ciailahean, M.H. 1988. Reguliatia tvetenia vissih rastenii.Moskva, Nauka: 554 p.

Collett, C.E., N.G. Harberd, and O. Leyser. 2000. Hormonal interactions in the control of Arabidopsis hypocotyls elongation. Plant Physiol. 124: 553-561.

Dmitreva,A.H., and M. Ploleacov. 1971. Necotorie biohimiceskoe osobennosti protessov vostonovlenia fertilnisti pilti u pastenii cucuruzi s CMS, In Ghenetica i Selectia na Ucraine, p.1. Kiev: 157p.

Duca, M., 1998. Physiological and genetical aspects of ASC-Rf system at *Helianthus annuus* L. Abstract of Dr.hab. thesis in biology. Chisinau, p.1- 40.

Duca, M.V., and A.I. Port. 2002. Physiological indices variation during different sunflower genotypes ontogenesis (*Helianthus annuus L.*). Romanian AN. I.C.C.P.T. 69:232-243.

Duca, M., G. Duca, and O. Budeanu. 1997. Fitohormones identification method. BOPI. Brevet (MD), Nr. 788.

Duca, M., A. Port, and T. Rotaru. 2003. Influence of diverse factors on the variability in auxin and gibberellin contents in *Helianthus annuus* L. Helia 26: 121-126.

Egorov, I.V., V.I. Sutulova, and I.N Livova. 1990. Izmenenie pola rastenii razlicinih sistematiceschih grupp pod deistviem reguleatorov rosta. p. 73-87. In: Reguleatori rosta rastenii, Moscow, Russia.

Fadeeva, T.S., S.P. Sosnihina, and H.M. Ircaeva. 1980. Sravnitelinaea ghenetica rastenii. Leningradskogo universiteta, Leningrad (St. Petersburg), Russia, 234 p.

Frenchel, P., and A. Galun. 1982. Mehanisma opilenia, rasmnojenia i selectia rastenii. Kolos, Moscow, Russia, 383 p.

Inoue, T., M. Higuchi, Y. Hashimoto, M. Seki, and M. Kobayashi. 2001. Identification of CRE 1 as cytokinin receptor from Arabidopsis. Nature 409:1060-1063.

Jacobsen, J.V., F. Gubler, and P.M. Chandler. 1995. Gibberellin action in germinated cereals. p. 246-271. In: P.J. Davies (ed.), Plant Hormones, Boston, MA, USA.

Kaul, M.L. 1988. Male sterility in higher plants. p. 364-370. In: Monographs on Theoretical and Applied Genetics, vol. 10. Springer-Verlag, New York.

Luis, J.P., and B. Durand. 1978. Studies with dioecious angiosperm *Mercurialis annua* L. (2n=16). Correlation between genetic and cytoplasmic sterility, sex segregation and feminizing hormones (cytokinins). Mol. Gen. Genet. 165:309-322.

Muthukrishanan, S., G.R. Chandra, and E.S. Maxwell. 1984. Hormonal control of a-amylase gene expression in barley. J. Biol. Chem. 258:13637-13639.

Nakajima, M., I. Yamaguchi, S., Kizawa, N. Murofushi, and N. Takahashi. 1991. Semi-quantification of GA_1 and GA_4 in male sterile anthers of rice by radiommunoassay. Plant Cell Physiol. 32: 511-513.

Rastogi, R., and V.K. Sawhney. 1990. Poliamines and flower development in the male sterile stamenless-2 mutant of tomato (*Lycopersion esculentum* Mill.). I. Levels of polyamines and their biosynthesis in normal and mutant flowers. Plant Physiol. 93:493-445.

Roni, A., M. Tollier, and B. Monties. 1990. The role of auxin and gibberellin in controlling lignin formation in primary phloem fibers and in xylem of *Coleus blumei* stems. Plant Physiol. 2:1743-1754.

Ross J. O., and D. Neill. 2001. New interactions between classical plant hormones. Trends Plant Sci. 6:2-4.

Simonenko, V.K., and E.V. Carpovici. 1982. Citologhiceschoie proevlenie raslicinih tipov mujckoi sterilnosyi u podsolnicinica. N.T. Biul. VSTI 34:34-41.

Smart, C.J., M. Francoise, and J. Christopher. 1994. Cell/Specific regulation of gene expression in mitochondria during anther development in sunflower. Plant Cell 6:11-825.

White, C.N., W.M. Proesbsting, P. Hedden, and C.J. Rivin. 2000. Gibberellins and seed development in maize. I. Evidence that gibberellin/abscisic balance governs germination versus maturation pathways. Plant Physiol. 122:1081-1088.

Zentella, R., D. Yamauchi, and T.H.D. Ho. 2002. Molecular dissection of the gibberellin/abscisic acid signaling pathways by transiently expressed RNA interference in barely aleurone cells. Plant Cell 14:2289-2301.