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GENETIC-PHYTOHORMONAL INTERACTIONS IN MALE FERTILITY AND MALE STERILITY PHENOTYPE EXPRESSION IN SUNFLOWER (*Helianthus annuus* L.)

Communication 2. IAA/GA_3 QUANTITATIVE RATIO OF SOME SUNFLOWER GENOTYPES REPRESENTING A *cms*-Rf SYSTEM

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SUMMARY

Quantitative ratio of IAA/GA_3 has been studied during the growth and development of different sunflower genotypes that represent a *cms*-Rf system. It was shown that the IAA/GA_3 ratio was variable and it depended on ontogenetic stage, plant organ and genotype. Thus, the IAA/GA_3 ratio the maximal value in the male sterile line while the minimal ratio was found in the restorer line RW 637 Rf. The highest IAA/GA_3 ratio was registered in cotyledon leaves, which decreased subsequently in the course of ontogenesis. During reproductive stages, the ratio was higher in inflorescences than in leaves. The exogenously applied gibberellic acid (GA_3) increased the ratio in the fertile line. Specificity of the IAA/GA_3 balance in the male sterility-fertility phenotype expression and in GA-induced pollen sterility is discussed.

Key words: ***cms*-Rf system, *Helianthus annuus* L., IAA/GA_3 ratio, male sterility, male fertility**

INTRODUCTION

Hormonal regulation of plant growth and development including interactions between different classes of hormones remains an important research topic in biology. Plants growth regulators, endogenous ones or those exogenously applied, are involved in male reproductive development, regulating sex differentiation (Ciailahean, 1988) and male (genetic and cytoplasmic) sterility promotion (Luis and Durand, 1978; Kaul, 1988; Rastogi and Sawhney, 1990; Nakajima *et al.*, 1991) in various species. Our previous work showed that sunflower *cms* lines contain lower amounts of gibberellins than fertile genotypes, including homozygous line with Rf

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genes (Duca, 2008). The quantitative ratio of auxins and gibberellins suggests their interactive role in microsporogenesis processes.

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

To study the functional role of IAA/GA_3 balance in the male sterility-fertility phenotype expression, their quantitative ratio was studied during the growth and development of different sunflower genotypes, which represent a *cms*-R*f* system.

MATERIALS AND METHODS

Material and methods were given in Communication 1 (Duca, 2008). In this work, for comparative studies, one *cms* line of sunflower *(Helianthus annuus* L.*)*, SW 501 *cms*, was additionally used.

RESULTS AND DISSCUSION

In our previous work it was shown that IAA and GA_3 contents in vegetative and reproductive tissues are variable and that they depend on ontogenetic stage, plant organ and genotype (Duca, 2008; Duca and Port, 2002; Duca *et al.*, 2003). However, the established changes in IAA and GA_3 concentrations were insufficient to reveal their functional role in the male fertility-sterility phenotype expression. For this purpose, the IAA/GA_3 ratio was analyzed during ontogenesis of sunflower plants using the *cms*-R*f* system.

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

Genotype	Stage of plant growth and development				
	Cotyledons	First pair of leaves	Flower bud stage	Active growth	Flower stage
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 R*f*	7.9	3.3	2.9	2.8	3.8
SW 501 *cms*	-	6.6	7.4	5.5	3.6

The highest IAA/GA_3 ratio was found in cotyledon leaves, with maximal values higher than 9 (Table 1). The line RW 637 R*f*, in contrast to all other studied lines, had the minimal lowest ratio, 7.9. This resulted exclusively from a high gibberellin content, because no obvious genotypic differences were observed in the auxin content (Duca, 2008). The IAA/GA_3 ratio two times lower during the stage of the first pair of true leaves and its values subsequently decreased during ontogenesis up to the lowest values, 2.5-3.6, found at the flower stage. The heterozygous F_1 hybrid with restored male fertility and self-pollinated homozygous male fertile lines showed almost similar values of the IAA/GA_3 ratio, while male sterile plants showed the highest ratio (Table 1).

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

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

Genotype	Reproductive stage		
	Flower bud stage	Active growth of inflorescence	Flower stage
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 R*f*	3.3	3.9	5.1
SW 501 *cms*	10.3	10.1	6.9

Significant results were recorded for the line RW 637 R*f*, which showed lowest values of this ratio both in leaves and in inflorescence assayed at the flower bud and active growth of inflorescence stages, when flower development and microsporogenesis occur.

Similar features of variable ratio were also determined for disk flowers (Table 3). It is thus evident that, during flower development, the values of the studied index decreased in the male sterile lines. But, in comparison with the male fertile genotypes, the IAA/GA_3 ratio in sterile flowers was higher in archesporogenesis and sporogenesis phases. Meanwhile, in the subsequent reproductive stage, carpogenesis, the values of IAA/GA_3 ratio were practically identical in all genotypes studied.

Table 3: IAA/GA_3 ratio in disk flowers of different sunflower genotypes

Genotype	Microsporogenesis stage		
	Arhesporogenesis	Sporogenesis	Carpogenesis
F_1	4.7	5.0	5.1
MB 514	4.1	4.8	5.0
MB 514 *cms*	8.9	6.6	4.6
MB 514 + GA_3	6.9	6.0	5.0
RW 637 R*f*	5.4	5.1	4.5
SW 501 *cms*	11.7	7.4	4.4

A particularly interesting physiological and genetic aspect of this study is the variation of the IAA/GA_3 ratio in the isogenic lines under exogenous gibberellins treatment (Table 4).

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

Line		Post-treatment period, hours						
		0	24	48	72		96	
					Leaves	Inflorescences	Leaves	Inflorescences
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
MB 514 *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 the flower bud stage increased the values of IAA/GA_3 ratio in leaf and inflorescence tissues of the male fertile lines almost to the level found in the line MB 514 *cms*. This increasing effect was significant after 72 and 96 h post-treatment. Variations in hormonal balance were not observed in the *cms* plants (Table 4). Although these genotypes are considered as hormone sensitive, the effect of GA treatment on endogenous IAA/GA_3 ratio was different. A similar effect of "genotype correction", back to the normal hormonal status under exogenous phytohormone influence, was also reported for several mutants with altered hormonal metabolism (Fadeeva *et al.*, 1980).

The following analysis of IAA/GA_3 ratio at the entire plant level (Figure 1) provided information on genetic and physiologic interactions which take part in self-regulation of the *cms*-R*f* system of sunflower (Figure 1).

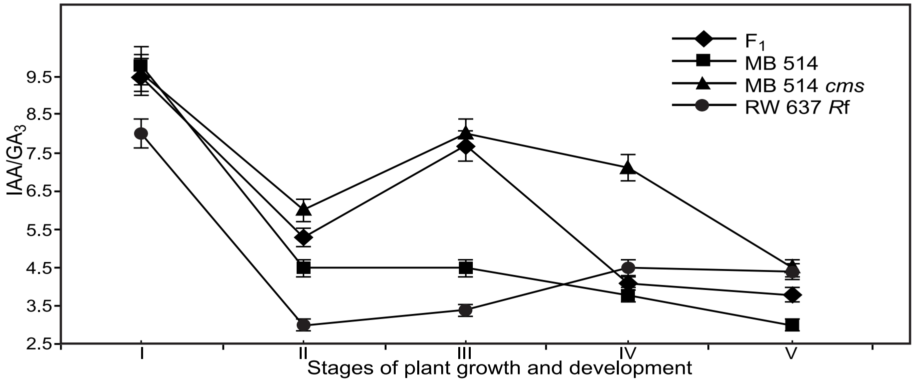

Figure 1: Hormonal ratio of different sunflower genotypes at the following stages of growth and development: I-cotyledons; II-first leaves; III- flower bud stage; IV-active growth; V-flower stage.

Thus, most significant differences were revealed at the flower bud stage. From the physiological point of view, it was shown that sunflower microsporogenesis and microgametogenesis occur prior to the beginning of flowering, when the diameter of the inflorescence bud reaches 2.5-4.5 cm (Smart *et al.*, 1994). The high auxin/gibberellin ratio found at the flower bud (7.9) and active growth of inflorescence (6.9) stages for the line MB 514 *cms* characterized by Srfrf genotype and the significantly lower ratio for RW 637 R*f*-FRfRf and MB 514-Frfrf suggest that the value of the hormonal ratio in the *cms* line was much above the optimal balance, which, according to our results, is approximately 4 for male fertile genotypes. The F_1 hybrid (SR*f*) contains sterile cytoplasm with nuclear R*f* genes, which restore male fertility in homozygous and heterozygous combination, resulting in the normalization of physiological and biochemical processes in plants (Dmitreva *et al.*, 1971). The fertility restorer genes present in the genotype of these plants apparently decrease the IAA/GA_3 ratio at the active growth and flower stages. Low values of this hormonal ratio are characteristic for fertile genotypes and high values for sterile ones. The hormone ratio alterations observed at the critical stages of reproductive development, especially during the microsporogenesis phase, indicate the phytohormonal mechanism of the *cms*-R*f* genetic system control, because in F_1 the ratio of analyzed phytohormones is already restored at the next stage of growth and development.

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

Our results indicate the occurrence of structural changes as a result of different auxin and gibberellin contents and their ratio. Therefore, the male sterile genotypes are characterized by high IAA/GA_3 ratio. Also, the GA_3 treatment of fertile plants, which resulted in phenotypic male sterility, induced an increase of the IAA/GA_3 ratio caused by the augmentation of endogenous auxins and gibberellins amount, which finally lead to a ratio approximately the same as in the male sterile genotypes (Table 4).

It appears that the hereditary cytoplasmic and GA-induced male sterility can be explained by the change in the phytohormone ratio and not in the concentration of phytohormones. It can be supposed that phenotypic expression of the morphogenetic program, especially microsporogenesis realization, depends on the IAA/GA_3 ratio. The hormonal balance has an essential role during the key stages of microsporogenesis-flower bud stage and active growth of inflorescence.

The above conclusions are sustained by the reported data. Thus, it was established that the IAA/GA_3 ratio regulates the primary differentiation of conductive fascicles and if the 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 sporophytic tissues, because mononuclear microspores of the tetrads develop normally till the stage of binuclear pollen (Simonenko, 1982). This process is characterized by the breakdown of interaction between anther nests and parenchymal tissues of the receptacle and insufficient provision of nutritive substances

(Frenchel, 1982), that ultimately causes tapetum tissue degeneration and disruption of pollen formation (Roni *et al.*, 1990).

CONCLUSIONS

The hormonal balance and interactions between various plant hormones as well as the cell capacity to receive hormonal signals are essential in the spatial and temporal physiological regulation of ontogenesis. The analysis of the IAA/GA_3 ratio during ontogenesis of sunflower plants using the *cms*-R*f* system made it possible to come to the following conclusions.

- The highest IAA/GA_3 ratio was found in cotyledon leaves. The value decreased subsequently in the course of ontogenesis to the lowest values (2.5-3.6) found at the flower stage.
- The heterozygous F_1 hybrid with restored male fertility and the self-pollinated homozygous male fertile lines showed similar values of the IAA/GA_3 ratio, while the male sterile plants registered highest hormonal ratios. Similar features of variable hormonal ratio were observed in both vegetative and reproductive tissues.
- The GA_3 exogenously applied at the flower bud stage increased the values of the IAA/GA_3 ratio in leaf and inflorescence tissues of the male fertile lines almost to the level found for the line MB 514 *cms*.
- Alterations in the hormonal ratio were significant at the microsporogenesis stage, which reveal the phytohormonal mechanism of the *cms*-R*f* 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 specificity of IAA/GA_3 balance in the male sterility-male fertility phenotype expression and in GA-induced pollen sterility is discussed.

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INTERACCIONES GENÉTICO-FITOHORMONALES EN LA EXPRESIÓN FENOTÍPICA DE LA ANDROFERTILIDAD Y ANDROESTERILIDAD EN GIRASOL (*Helianthus annuus* L.)

Parte II: Relación cuantitativa IAA/GA_3 de algunos genotipos de girasol que representan un sistema *cms*-R*f*.

RESUMEN

Se estudió la relación cuantitativa IAA/GA_3 durante el crecimiento y desarrollo de diferentes genotipos de girasol que representan un sistema *cms*-R*f*. Se ha demostrado que la relación IAA/GA_3 es variable y depende de los estados ontogénicos, órganos y genotipo. Así la relación IAA/GA_3 presentó valores máximos para la línea androestéril y mínimos para la línea restauradora RW637R*f*. La más alta relación IAA/GA_3 se registró en hojas cotiledonares que decrecieron subsecuentemente durante la ontogénesis. La relación hormonal en estadíos reproductivos fue mayor en inflorescencias que en hojas. El ácido giberélico (GA_3) aplicado en forma exógena incrementó la relación hormonal en la línea fértil. Se discute la especificidad del balance IAA/GA_3 en la expresión de los fenotipos androfértiles y androestériles y en la esterilidad de polen inducida por GA.

L'INTERACTION ENTRE LES SYSTÈMES GÉNÉTIQUE ET PHYTOHORMONALE DANS LA RÉALISATION PHETOTIPIQUE DE L'ANDROSTÉRILITÉ-ANDROFERTILITÉ CHEZ LE TOURNESOL *Helianthus annuus* L.

Partie II: L'étude de rapport entre les auxines et gibbérellines chez les génotypes du tournesol qui détermine la stérilité cytoplasmique masculine

RÉSUMÉ

On a réalisé l'étude du rapport entre la quantité des auxines et gibbérelline dans le processus du croissance et développement des différents génotypes de tournesol qui constituent le système de la stérilité cytoplasmique masculine. Les résultats des recherches ont montré que le rapport entre les auxines et les gibbérellines est dépendant de la phase ontogénétique, les organes étudiés et le génotype de tournesol. La ligne androstérile est caractérisée par un rapport maximal entre les auxines et gibbérelline pendant que la ligne RW 637 R*f* est caractérisée par un rapport minimal. L'analyse de ce paramètre a montré que le rapport entre les auxines et gibbérellines est plus élevé dans les inflorescences que dans les feuilles. La valeur plus grande de rapport auxines/ gibbérellines a été enregistré dans les feuilles de cotylédon. On atteste une diminution du rapport dans l'ontogenèse. Chez les lignes fertiles le traitement avec les gibbérellines cause une croissance du rapport auxines/ gibbérellines. On dispute sur la problème vis-à-vis du rapport spécifique auxines/ gibbérellines dans la manifestation phénotypique de l'androstérilité- androfertilité, ainsi que dans l'induction phénotypique de la stérilité de du grain de pollen par le traitement par gibbérellines.