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Effect of a Signaling System of Plant on Gene Interaction in Inheritance of Root System Characteristics of Arabidopsis Thaliana (L.) Heynh

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ABSTRACT

Results of a study of the plant signalling system interconnection and gene interaction in inheritance of characteristics of Arabidopsis root system are presented. On the basis of the relationship between the signaling system of the plant and the interaction of genes in the inheritance of the signs, the concept of the mechanism of interaction of genes has been improved. Gene interaction problem is closely related to the plant development regulation signalling system. Mechanism involved in gene interaction may be explained based on current idea of molecular principles of biological response. Genes controlling signalling paths and causing development of a characteristic or a response may be conventionally divided into two large groups: 1. genes responsible for signal perception and transmission inside the cell; 2. genes directly ensuring implementation of response to a signal, i.e. so-called primary and secondary response genes. Genes from the first group encode protein receptors that percept a certain signal of chemical or physical origin, including special proteins (for example, G-proteins, protein kinases, protein phosphatases), some low-molecular compounds included into the cascade system of messenger molecules that transmit the perceived signal into the cell nucleus. Genes from the second group control transcription factors that regulate expression of certain genes, and response to the signal. Affected by mutations occurring in various genes that control certain links of the signalling chain, signalling path to the cell nucleus and response are blocked partially or in full which leads to distortion in manifestation of the characteristic on the plant level in general or its organ level. Such phenomenon is observed in realization of many characteristics in animals and plants, including in A. thaliana.It is established that recessive epistasis (alf3-1 alf3-1 > CTR1_) is observed in the second crossing generation for plants of ctr1-1 x alf3-1 mutant lines. When nph4-1 x iar2-1 mutant-line plants are crossed, polymeric interaction of genes NPH4 and IAR2 occurs in F2 generation.

Keywords: *Arabidopsis thaliana (L.) Heynh., root system, gene, mutation, gene interaction, signalling system.*

INTRODUCTION

In the first half of the 20th century during development of classic genetics, the main genetic principle was an idea about the fact that one gene forms one characteristic (Lobashev, 1985). To the extent of accumulation of knowledge concerning molecular and genetic mechanisms of organism growth and development processes, there were discovered no or very few monogenic characters, display whereof is determined by one gene, in nature, and gene-character ratio is far more complicated. Most of characters are polygenic and determined by a larger quantity of genes. Phenomena of pleiotropic gene action and nonallelic gene interaction (complementarity, epistasis, polymery, modifying effect of genes) (Dubinin, 1970). However, gene interaction mechanism which impacts the nature of segregation of variously crossed hybrids hasn't been studied sufficiently. Those examples of inheritance of characteristics in gene interaction which are presented in classical textbooks on Genetics are provided without understanding of molecular genetic processes of development regulation and cannot explain gene interaction mechanism. At the same time, exclusive of molecular genetics, biochemistry and physiology, a genetic analysis of inheritance of characteristics in gene interaction taken by itself cannot reveal nature of this interaction. It is obvious that the final conclusion concerning

mechanism of gene interaction may be made only after a picture of gene interaction in a genetic system on biochemical and molecular levels is revealed.

Lately, due to rapidly developing studies of molecular mechanisms of gene expression regulation, it is growing clearer and clearer that gene interaction mechanism problem is closely related to cellular signalling systems (Inge-Vechtomov, 2000; Hablak, Parii, 2013). Existence of signalling chains that using specific protein receptors in most cases located in cell membrane accept signal impulses, transform, amplify and transmit them to cell genom causing gene expression re-programming and metabolism (including dramatic) modulation, related to activation of previously "silent" and inactivation of some active genes was discovered in plant cells (Tarchevsky, 2002).

Achievements in study of plant genom, recovery of genes responsible for certain stages of plant growth, development, ageing, their response to stresses and pathogens have been increasing in the last decade. Genes controlling regulatory systems of plants starting from receptor proteins and finishing with genes of factors determining activation of certain genetic programs have been recovered (Kulayeva, 2000).

So far, molecular, genetic and physiological studies of *A. thaliana* mutants have enabled to isolate and sequence a wide range of genes controlling certain links of the signalling chain. They include *AGB1*, *AHK2*, *ERS1*, *GPA1*, *CTR1*, *ALF3*, *NPH4*, and *IAR2*.

AHK2 and *ERS1* genes encode histidine kinases which are membrane receptors (Hua et al., 1995; Liu et al., 2010; Riefler et al., 2006). *GPA1* and *AGP1* genes control alpha and beta-subunits of heterotrimeric GTP-binding proteins (G-proteins) responsible for hormonal signal transmission from serpentine-type receptors to transcription factors (Ma et al., 1990; Okamoto et al., 2001). *CTR1* gene encodes *CTR1* protein (signalling repressor) belonging to a family of serine/threonine protein kinases prevalent in eucaryotes participating in so-called MAP-kinase cascade (An et al., 2010). *ALF3*, *NPH4* and *IAR2* genes control transcription factors regulating gene expression (Di Donato et al., 2004).

At the same time, effect of the plant development regulation signalling system on interaction of these genes in inheritance of characteristics of Arabidopsis root system haven't been studied so far which was a cause for our studies.

Materials And Methods

The study materials were *Arabidopsis thaliana* (L.) Heynh. plants of Columbia (Col-O) ecotype (race) and mutant lines *ahk2-5 (arabidopsis histidine kinase 2-5), agp1-2 (arabinogalactan-protein 1-2), ers1-2 (ethylene response sensor 1-2), gpa1-3 (g protein alpha subunit 1-3), ctr1-1 (constitutive triple response 1-1), alf3-1 (aberrant lateral root formation 3-1), nph4-1 (non-phototrophic hypocotyl 4-1), iaa2-1 (iaa-alanine resistant 2-1).* Seeds of the mutant lines were obtained from Nottingham Arabidopsis Stock Centre (NASC), UK and Arabidopsis Biological Resource Centre (ABRC), USA.

Plants were grown in the laboratory in an aseptic culture in tubes on Knop's agar medium enriched with microelements (Rubin et al., 1978).

Seeds were prepared for planting by means of jarovization for 5 days at 4–6^0 C and further one-day germination at room temperature. The tubes were wrapped with two layers of paper to protect from heating and exposure of plant roots to light. The plants were cultivated at 18–20^0 C, illumination was 24 hours within 4000–7000 lux.

Castration and forced hybridization were performed under MBS-9 type microscope. Genetic analysis of inheritance of plant root system characteristics was conducted in F_1, F_2. Selection scope in the second generation made up 196 plants. For observation over the plants, they followed generally-accepted methods of vegetation and comparative-morphological studies (Dospekhov, 1985).

Results And Discussion

Any phenotypic characteristic of the plant is a result of functioning of the signalling system, major components whereof are usually considered to be protein receptors accepting a signal, secondary mediators transmitting the accepted signal to the cell nucleus, and transcription factors regulating gene expression and response to the signal.

As a rule, mutations in genes coordinating certain links of the signalling chains, cause modulation of the plant characteristics. Table 1 presents results of studies on root system structure in Arabidopsis mutant-line plants

carrying mutations across genes that control intracellular signalling path. Data in the table show that mutations in these genes affect the quantity and length of roots in the plant root system in various ways. Mutations *agb1-2*, *ahk2-5*, *ers1-2* across genes *AGB1*, *AHK2*, *ERS1* cause increase in orders of roots. In such cases, plants affected by *agb1-2*, *ahk2-5*, *ers1-2* mutations form larger number and length of lateral roots of the first and further orders of roots comparing to the original Col-O race.

At the same time, *gpa1-3*, *ctr1-1*, *alf3-1*, *nph4-1*, *iar2-1* mutations in *GPA1*, *CTR1*, *ALF3*, *NPH4*, *IAR2* genes precondition decrease in extent of root branching in the root system. In these cases, *gpa1-3*, *ctr1-1*, *alf3-1*, *nph4-1*, *iar2-1* mutant plants have a lesser number and length of lateral roots of various root orders comparing to Col-O ecotype.

It should be noted that *alf3-1*, *gpa1-3* mutations of *ALF3*, *GPA1* genes result in the plant root system type alteration. It is known that wild-type Arabidopsis widespread in nature develops a mixed-type root system (Hablak, Abdullaieva, 2010). Mutation *gpa1-3* across *GPA1* gene causes formation of a taproot system in plants. Mutation *alf3-1* in *ALF3* gene preconditions in plants formation of a primary root only that usually doesn't branch onto lateral roots. In these cases, notions of a root and a root system coincide.

Taking into account unclarity of a problem of interconnection of plant development regulation signalling system and gene interaction in inheritance of characteristics, we performed a number of crossings between mutant Arabidopsis plants having mutations of genes encoding primary links of the signalling chain in their genotype.

For Arabidopsis, *ctr1-1* mutant line plants have decreased extent of root branching, and *alf3-1* mutant line plants do not have secondary and lateral roots of the primary root, i.e. form only a primary root. Dominant allele *CTR1* preconditioning normal length of lateral roots prevails over *ctr1-1* allele which determines their shortened value. The other allelic pair located on the other pair of homologous chromosomes determines existence of secondary and lateral roots of the primary root. This particularity is regulated by the dominant allele *ALF3*. Recessive allele *alf3-1* determines their absence.

Table1. *Average values of biometric parameters of root system characteristics in Col-O ecotype and mutant lines affecting root branching, during bud stage (on 30th day after seed germination)*

Code of the line	Type of roots								Total roots
	Primary root		Lateral roots of the primary root		Secondary roots		Lateral roots of secondary roots		
	NR pcs	LR mm	NR pcs	LR mm	NR pcs	LR mm	NR pcs	LR mm	
WT (Col-0)	1	39.1	29.6	12.5	1.1	7.5	10.3	6.6	42
mutations in genes encoding protein receptors									
ahk2-5	1	47.0	45.0	22.4	2.4	10.1	19.6	13.9	68.0
ers1-2	1	50.4	55.2	36.4	2.7	11.9	23.3	20.4	82.2
mutations across genes controlling secondary mediators									
gpa1-3	1	31.9	16.3	7.4	0	0	0	0	17.3
agb1-2	1	64.7	45.6	25.4	6.3	14.3	15.7	12.5	68.6
ctr1-1	1	30.5	6.4	4.8	0.8	5.5	3.2	2.3	11.4
mutations of genes encoding transcription factors									
alf3-1	1	29.4	0	0	0	0	0	0	1
nph4-1	1	31.4	12.3	6.1	1.0	3.2	5.2	3.6	19.5
iar2-1	1	31.1	14.9	5.9	1.0	4.2	5.7	3.4	22.6
Least significant difference (LSD_{05}), pcs/mm	-	3.54	2.0	1.19	0.58	1.14	1.25	0.86	2.79

Note: NR - number of roots, LR - length of roots.

When plants from mutant lines *ctr1-1* and *alf3-1* are crossed, first-generation hybrids *CTR1 ctr1-1 ALF3 alf3-1* develop normal lateral roots of the primary root, and secondary roots. In the second generation, self-pollination of such plants results in segregation into three phenotype classes in the following ratio: 105 with typical secondary and lateral roots of the

primary root, 31 with their reduced length, 44 without secondary and lateral roots of the primary root.

Statistical evaluation of differences performed between the experimental and theoretically expected results of segregation in F_2 generation using fitting criterion χ^2 showed that hypothesis of segregation according to 9:3:4 scheme was confirmed (table 2).

Table 2. *Segregation in F2 generation on CTR1 and ALF3 genes*

Code	*CTR1_ ALF3_*	*ctr1-1 ctr1-1 ALF3 _*	*CTR1_ alf3-1 alf3-1;ctr1-1 ctr1-1 alf3-1 alf3-1*	Total
f	105	31	44	180
f^1	101	34	45	180
d	4	-3	-1	
d^2	16	9	1	
χ^2	0.16	0.26	0.02	0.44

These results may be explained by recessive epistasis of *alf3-1 alf3-1 > CTR1_* type when a recessive allele of one gene - *ALF3* in a homozygous state suppresses influence of the dominant allele of the other gene - *CTR1* in a homo- or heterozygous state.

With significant change in segregation in F_2, inheritance of characteristics of the root system takes place in further crossing of plants of mutant lines *nph4-1* and *iar2-1*.

In Arabidopsis, development of the normal length of lateral roots of the primary root is determined by several dominant genes - *NPH4*, *IAR2* and others, and the shortened length - by recessive genes *nph4-1*, *iar2-1* and so on. When two plants of mutant lines *nph4-1* and *iar2-1* with reduced extent of root branching, F1 hybrids with normal length of lateral roots of various orders are formed. In the second generation of such crossing, 15/16 of all plants turn out to have a varying length of lateral roots and 1/16 has no lateral roots (table 3). This fact may be explained by effect of polymeric interaction of genes *NPH4* and *IAR2* on development of *"lateral root length"* characteristic.

Table3. *Segregation in F_2 generation on NPH4 and IAR2 genes*

Code	*NPH4 _ IAR2 _; NPH4_ iar2-1 iar2-1; nph4-1 nph4-1 IAR2 _*	*nph4-1 nph4-1 iar2-1 iar2-1*	Total
f	172	12	184
f^1	173	11	184
d	-1	1	
d^2	1	1	
χ^2	0.005	0.09	0.095

In general, obtained study results show that the gene interaction mechanism is closely related to modern idea of molecular principles of biologic responses. Development of any characteristic, property or reaction to unfavourable environmental conditions in plants is resulted from functioning of many genes that may interact in various ways. Expression regulation of these genes is controlled by endogenous and exogenous signals. They are received by specific receptors and transmitted through mediator molecules on a series of transcription factors suppressing or initiating transcription of certain genes causing the response.

Affected by mutations occurring in various genes controlling certain links of the signalling chain, signalling path to the cell nucleus and the response are blocked partially or in full which leads to distortion in manifestation of the characteristic on the plant level in general or its organ level. Such phenomenon is observed in realization of many characteristics in animals and plants, including in *A. thaliana*. When such characteristics are inherited, plants display all main forms of gene interaction.

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