Cloning of Glycine-rich RNA-binding Proteins Genes from Different tobacco Germplasm Resources and Expression Analysis under Different Abiotic Stresses

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ABSTRACT

Glycine-rich RNA-binding proteins (GR-RBPs) play important roles in the post-transcriptional regulation. In this paper, 11 GR-RBPs genes (RGP-3) were isolated and sequenced from different tobacco varieties including cultivated tobacco and wild tobacco, Sequence homology, phylogenetic analysis and gene expression were discussed as emphases. Sequences alignment revealed that RGP-3 in different tobacco varieties show high homology, with 100% similarity between K326 and *N. sylvestris* and the lowest similarity of 88.1% between *N. goodspeedi* and *N. repanda*. There were 426 variation sites in *RGP-3* genomic sequence, but 383 sites were located in introns. Phylogenetic trees revealed that K326 Hongda were evolutionarily closest to *N. sylvestris*, suggesting closest genetic relationship between *N. tabacum* and *N. sylvestris*. Expression analysis revealed that *RGP-3* in *N. tabacum* was strongly induced by water, weakly induced by SA, and unaffected by ABA treatment. These results will provide the basis for wide cross to improve tobacco biotic and abiotic resistance.

INTRODUCTION

More and more plant glycine-rich RNA binding protein are isolated from plants, such as corn, spruce, arabidopsis , tobacco , barley, crop brassicas, and sweet cherry. It was reported in the previous studies that a lot of stress factors including chilling, injury, drought, allergic reaction, ABA treatment, SA treatment and stress would effectively lead to a significant effective increase in the level of RNA in the GR-gene family.Based on the results of stress analysis, more and more studies were carried out in the light of specific physiological functions of GR-RBPs. For example, LpGRP1 of rye involved in the process of control over chilling and *AtGRP2* of arabidopsis related to the control of seed germination and plant growth under the chilling circumstances. Five types of gene, including *NsRGP-1a*、*NsRGP-1b*、*NsRGP-1c*、*NsRGP-2* and *NsRGP-3*, were obtained trough the isolating process based on *N. sylvestris* in terms of tobacco while GR-RBPs gene in tobacco has been reported rarely. In this paper *RGP-3* in tobacco was studied, which in 11 different types of tobacco were cloned and compared, and which in *N. tabacum* was cDNA cloned and genetic expression analyzed. These works will contribute for the further research of evolution and genetic function of GR-RBPs.

Table1. The length of *RGP-3*in different tobacco varieties

Acesssion	Origin	The length of *RGP-3* gene(bp)	Acesssion	Origin	The length of *RGP-3* gene(bp)
N. tabacum (K326)	USA	1242	*N.repanda*	NA	1222
N.tabacum(Hongda)	CHINA	1243	*N. rustica*	SA	1230
N. sylvestris	SA	1243	*N.clevelandii*	NA	1227
N. glauca	SA	1243	*N. undulata*	SA	1222
N. goodspeedi	Austrilia	1212	*N. alata*	SA	1233
N . plumbaginifolia	SA	1240			

Fig. 1. Diagram of genomic sequences of *RGP-3* between *N. sylvestris* and *N. tabacum* (Hongda). ↑indicates base insert, ↓indicates base deletion

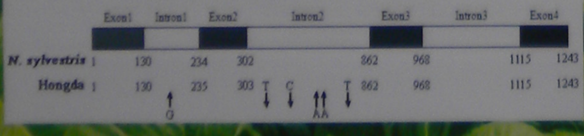


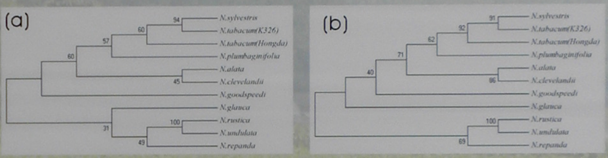
Fig 2 Identity analysis between 11 homologous genomic sequences of *RGP-3*

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11		
1	■	97.5	100.0	93.6	93.5	92.4	94.1	96.3	90.5	91.2	91.0	1	*N. tabacum* (K326)
2	133.3	■	97.4	92.9	92.3	91.2	92.3	94.5	89.9	90.3	90.9	2	*N. tabacum* (Hongda)
3	27.0	57.0	■	93.7	93.6	92.4	94.1	96.3	90.5	91.2	91.0	3	*N. sylvestris*
4	36.7	54.0	7.5	■	91.3	91.7	90.1	92.1	88.2	90.0	90.1	4	*N. alata*
5	34.5	61.8	8.0	6.9	■	91.2	89.6	92.3	88.2	88.7	88.5	5	*N. clevelandii*
6	34.1	61.1	8.1	8.0	8.6	■	92.1	91.8	90.0	90.5	90.6	6	*N. glauca*
7	32.8	62.0	7.4	9.9	10.2	8.0	■	93.0	88.1	90.0	89.2	7	*N. goodspeedi*
8	31.6	57.2	4.8	7.7	8.1	7.7	7.6	■	89.6	90.5	90.3	8	*N. plumbaginifolia*
9	36.7	63.9	10.8	11.6	11.7	10.0	11.8	11.0	■	88.6	88.5	9	*N. repanda*
10	35.9	58.6	8.5	8.6	9.5	8.3	8.8	8.3	10.3	■	98.4	10	*N. rustica*
11	37.6	59.1	9.3	9.1	10.2	8.7	9.7	9.0	10.5	1.6	■	11	*N. undulata*
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**		

Fig. 3. Phylogenetic tree of *RGP-3* genomic sequences (a) and introns (b) constructed by ML method



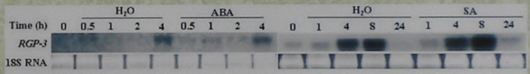


Fig. 4. Expression patterns of *RGP-3* during stresses. Water-treated leaves were made for contrast in ABA and SA treatment

CONCLUSION

- *RGP-3* genomic sequences showed a high level of homology among different tobacco germplasm resources, even the lowest identity between *N. goodspeedi* and *N. repanda* enjoy was 88.1%.
- It is well-known that *N. glauca* and *N. Rustica* belong to *Rustica*, and *N.sylvestris*, *N.plumbaginifolia* as well as *N.alata* belong to Petunioides Alatae. In this study, *N. glauca* was close to *N. Rustica*, and *N.sylvestris* was close to *N.plumbaginifolia* and *N.alata*, which was in accordance with traditional classification. Based on the evolution analysis of RGP-3, the genetic distance between *N. tabacum* and other 8 wild species was farther. So, these wild species could be exploited as the breeding parents to widen the genetic of flue-cured varieties.
- Excellent variety of tobacco is an important genetic basis of leaf quality. Although K326 and Hongda are very popular in the domestic tobacco industry because of the unique quality, some diseases like black shank (*Phytophthora parasitica var.nicotianae*)and black root(*Thielaviopsis basicola*) make the industry headaches for a long time. However, *N. undulata* and *N. repanda*, which showed a relative lower level with K326 and Hongda, have large range of disease-resistance sources (including resistant to black shank and black root).So, it provides an useful information that wild species resources are used for distant hybridization to improve the disease resistance of cultivated tobacco.
- Expression analysis revealed that *RGP-3* in *N. tabacum* was strongly induced by water, weakly induced by SA, and unaffected by ABA treatment. Therefore, it could be inferred that *RGP-3* may perform some functions with ABA-independent pathway under stresses. It was shown that *RGP-3* in *N. tabacum* was strongly induced by water, weakly induced by 4-hour SA treatment, 8-hour SA treatment, 24-hour SA treatment, and unaffected by ABA treatment.