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R Krishna
Aryabhatta College, University of Delhi, Delhi, India

Chandan Bharti Mishra
Aryabhatta College, University of Delhi, Delhi, India

Correspondence
R Krishna
Aryabhatta College, University of Delhi, Delhi, India

Application of antisense RNA technology for crop improvement; A review

R Krishna and Chandan Bharti Mishra

Abstract
The naturally occurring antisense RNAs are untranslated transcripts which are small and consists of sequences complementary to a target mRNA and acts as a biological off switch. Antisense RNAs can cause target mRNA's complete degradation, change mRNA processing and/or regulate mRNA transcription and in this manner gene expression can be regulated or completely inhibited as it functions at post transcriptional level. This antisense RNA technology has very wide application and has proven to be an effective technique for understanding function/s of individual genes. This technique has been broadly used in Crop Improvement by down-regulating specific gene/s and completely inhibiting protein production responsible for damaging/ reducing crop production.

Keywords: Application RNA technology crop improvement

Introduction
Antisense RNA technology involves introduction of a construct with target gene cloned downstream to a promoter in a reverse orientation. Antisense RNA synthesized by this construct has a sequence complementary to the target gene sequence. The duplex so formed is cleaved by Dicer into short fragments of ~ 21 bp known as small interfering RNA (siRNA). SiRNA molecule incorporated in multi-protein complex is called RNA Induced Silencing Complex (RISC). One of the siRNA strand (passenger) is degraded upon RISC activation, the other strand (guide) remained in the complex (Matzke and Birchler, 2005, He and Hannon, 2004) [8, 4]. The activated RISC complex specifically binds to complementary target RNA and the Argonaute, a catalytic component of RISC complex degrade target mRNA (Matzke and Birchler, 2005) [8].

Crop improvement using Antisense RNA technology
Antisense RNA technology approach has been widely used for silencing target genes of various categories and functions including the modification of fatty acids composition in various seed oil crop like brassica and cotton. This technology has also been used in developing pathogens resistant transgenic plant, altering pigment production in flower and manipulating different metabolic pathways. This review will enlighten broad applications of antisense RNA technology in crop improvement.
Flavr savr tomato was the first crop developed by Calgene Company using antisense RNA technology in United States. The enzyme Polygalacturonase present in the tomato degrades pectin of the cell wall. This enzymatic degradation causes softening of fruit, making them susceptible to fungal infection. Antisense RNA technology was used to suppress polygalacturonase gene which slows down the ripening process, prevent it from softening and increases their self-life (Sheehy *et al*., 1988) [11]. In a separate study, Hamilton *et al.* (1990) [3] down regulated ethylene production in tomato plant to control fruit ripening and leaf senescence. Knock down of ACC-oxidase enzyme in transgenic tomato reduced the production of ethylene in fruit and wounded leaves. Ethylene production in fruit and wounded leaves was reduced by 87% and by 68% respectively as compared to control plant.
In *Brassica. rapa* and *Brassica napus,* low oleic acid transgenic lines were developed using antisense to stearoyl-CoA 9-desaturase (*SAD*) gene. Using this strategy stearic acid (18:0) content was increased by 2% to 40% in *B. napus*. Similarly in *B. rapa* seeds the stearic acid level rose from 1% to as high as 32% (Knutzon *et al.* 1992) [6]. The fatty acid composition of

zero erucic acid (C22:1) line of *Brassica juncea* (VH486) was modified using antisense RNA technology. Antisense mediated gene silencing against Fatty acid desaturase 2 (*FAD2*) gene was resulted in 73% oleic acid (C18:1) and 8-9% each of Linoleic (C18:2) and Linolenic acid (C18:3) in comparison to 53% oleic acid, 24% Linoleic Acid and 16% Linolenic acid in the parental line (Sivaraman *et al*. 2004) [12]. *FAD2* gene in *Brassica carinata* was down regulated using co-suppression and antisense approaches. Both methods resulted in transgenic line exhibiting decreased proportion of polyunsaturated fatty acids (Linoleic and Linolenic acid) and significantly increased proportion of Oleic acid (18:1), erucic acid (22:1) and total very long chain fatty acids (VLCFAs). Co-suppressed *FAD2 B. carinata* lines exhibited 3-18% decrease in Linoleic acid (18:2), 22-49% decrease in Linolenic acid (18:3) and significantly increased Oleic acid (36-99%), erucic acid (12-27%) and VLCFAs (6-15%) content. Transgenic *B. carinata* lines developed using an antisense *FAD2* approach exhibited decreased proportion of 18:2 and 18:3 (9-39% and 33-48%, respectively) and significantly increased oleic acid (54-130%), 22:1 (5-19%) and VLCFAs (6-21%) (Jadhav *et al*. 2005) [5].

Gossypium hirsutum cv Coker 315 (Cotton) has a high oleic desaturation proportion (ODP) value ranging from 0.80 to 0.085, meaning that over 80% of oleic acid produced in developing seed is subsequently converted to linoleic acid. Hairpin RNA- encoding gene constructs (HP) and antisense gene construct targeted against fad 2-1 gene were transformed in cotton cv coker 315. Transgenic plant carrying the *FAD2*-HP and *FAD2* antisense construct showed a considerable reduction in ODP value as low as 0.04, indicating a 95% down regulation of FAD2 gene activity (Liu *et al*. 2002) [7].

FAD2 gene in *Arabidopsis* was down regulated using antisense (AS), Hairpin (HP) as well as hairpin antisense (HPAS) constructs. Antisense suppression of *FAD2* resulted in an increase of 18:1 fatty acid from 15.2% to 44.2%. This level increased to 56.9% and 61.7% with *FAD2-HP* and *FAD2*- HPAS constructs (Nguyen and Shanklin 2008) [9].

Down regulation of *FAD2* gene expression in soybean was also achieved by antisense strategy. Fatty acid analysis of oil of T2 plant showed increment in oleic acid content from 14.4% to 28.2% with decrease in Linoleic Acid content from 31.9% to 19.5% (Gupta *et al*. 2009) [2]. In a separate study, increase of oleic acid content upto 56.7% was reported by inhibiting *FAD2-1* gene expression in soybean (Zhang *et al*., 2014) [15].

In rice (*Oryza sativa* L.) Lipooxygenase (LOX3) gene is responsible for lipid peroxidation, damages the endosperm upon storage. The expression of LOX3 gene in transgenic plant was suppressed using antisense RNA technology which resulted into decrease in accumulation of 9-hydroperoxyoctadecadienoic acid (9-HPOD). Reduced LOX3 activity in endosperm provided increase in grain storability and viability (Xu *et al*., 2014) [14].

Plant pathogens severely affect plant growth and productivity. Post transcriptional gene silencing technique has also been extensively used in raising various disease resistant transgenic plants. Cotton leaf curl virus (CLCuV) causes leaf curl disease in cotton and reduces their productivity worldwide. A CLCuV resistant cotton plant has been developed by transforming βC1 gene in antisense orientation using agrobacterium mediated transformation (Sohrab *et al*., 2016) [13]. In another report, A *Cotton leaf curl Burewala virus* (CLCuBuV) resistant tobacco transgenic plants have been developed using mi RNA techniques (Ali *et al*., 2013) [1]. Tomato resistant to Cucumber Mosaic Virus has been developed by RNA interference technology (Ntui *et al*., 2014) [10]. Cassava Mosaic Virus (CMV) is responsible for massive loss of cassava production in Africa (19.6- 27.8%). CMV resistant transgenic plant was developed by down-regulating *AC1*, *AC2* and *AC3* genes of Cassava Mosaic Virus using antisense RNA technology (Zhang *et al*., 2005) [16]. Similarly, RNA interference technology was used to develop Mungbean yellow mosaic India virus (MYMIV) resistant Cowpea crop by targeting *AC2* and *AC4* viral genes.

Conclusion

Antisense RNA technology has very wide application and offers endless scope in crop improvement. This technique can be used for increasing the productivity of various crops by identifying specific genes which control protein responsible for lowering the shelf-life of the crop and down regulating them. At the same time, it can also be used against the pathogens which are responsible for destroying crops and reducing the overall yield.

References

1. Ali I, Amin I, Briddon RW, Mansoor S. Artificial microRNA-mediated resistance against the monopartite begomovirus Cotton leaf curl Burewala virus. Virology journal 2013;10:231-238.
2. Gupta NC, Sinha SK, Jolly M, Dubey N, Sachdev A. Antisense RNA-mediated inhibition of *GMFAD2-1* encoding omega-6-desaturase. Indian Journal of Plant Physiology 2009;14:336-343.
3. Hamilton AJ, Lycett GW, Grierson D. Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. Nature 1990;346:284-287.
4. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nature Review Genetics 2004;5:522-531.
5. Jadhav A, Katavic V, Marillia E-F, Michael Giblin E, Barton DL, Kumar A *et al*. Increased levels of erucic acid in *Brassica carinata* by co-suppression and antisense repression of the endogenous *FAD2* gene. Metabolic Engineering 2005;7:215-220.
6. Knutzon DS, Thompson GA, Radke SE, Johnson WB, Knauf VC, Kridl JC. Modification of Brassica seed oil by antisense expression of a stearoyl-acyl carrier protein desaturase gene. Proceedings of the National Academy of Sciences of the United States of America 1992;89:2624-2628.
7. Liu Q, Singh SP, Green AG. High-stearic and High-oleic cottonseed oils produced by hairpin RNA-mediated post-transcriptional gene silencing. Plant physiology 2002;129:1732-1743.
8. Matzke MA, Birchler JA. RNAi-mediated pathways in the nucleus. Nature Reviews Genetics 2005;6(1):24-35.
9. Nguyen T, Shanklin J. Altering *Arabidopsis* oilseed composition by a combined antisense-hairpin RNAi gene suppression approach. Journal of the American Oil Chemists' Society 2008;86:41-49.
10. Ntui V, Kong K, Azadi P, Khan R, Chin D, Igawa T *et al*. RNAi-Mediated Resistance to Cucumber Mosaic

Virus (CMV) in Genetically Engineered Tomato. American Journal of Plant Sciences 2014;5:554-572.
11. Sheehy RE, Kramer M, Hiatt WR. Reduction of polygalacturonase activity in tomato fruit by antisense RNA. Proceedings of the National Academy of Science USA 1988;85(23):8805-9.
12. Sivaraman I, Arumugam N, Sodhi Y, Gupta V, Mukhopadhyay A, Pradhan A *et al*. Development of high oleic and low linoleic acid transgenics in a zero erucic acid *Brassica juncea* L. (Indian mustard) line by antisense suppression of the *FAD2* gene. Molecular Breeding 2004;13:365-375.
13. Sohrab SS, Kamal MA, Ilah A, Husen A, Bhattacharya PS, Rana D. Development of Cotton leaf curl virus resistant transgenic cotton using antisense ßC1 gene. Saudi journal of biological sciences 2016;23(3):358-362.
14. Xu H, Wei Y, Zhu Y, Lian L, Xie H, Cai Q *et al*. Antisense suppression of LOX3 gene expression in rice endosperm enhances seed longevity. Plant Biotechnology Journal 2015;13(4):526-39.
15. Zhang L, Yang XD, Zhang YY, Yang J, Qi GX, Guo DQ *et al*. Changes in oleic Acid content of transgenic soybeans by antisense RNA mediated posttranscriptional gene silencing. International journal of genomics 2014, 921-950.
16. Zhang P, Vanderschuren H, Fütterer J, Gruissem W. Resistance to cassava mosaic disease in transgenic cassava expressing antisense RNAs targeting virus replication genes. Plant Biotechnology Journal 2005;3(4):385-97.