BioScientific Review (BSR)

Volume 3 Issue 1, 2021

ISSN(P): 2663-4198 ISSN(E): 2663-4201

Journal DOI: https://doi.org/10.32350/BSR

Issue DOI: https://doi.org/10.32350/BSR.0301

Homepage: https://journals.umt.edu.pk/index.php/BSR

Article: **Kanamycin in Cereal Biotechnology: A Screening or a Selectable Marker?**

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Article DOI: https://doi.org/10.32350/BSR.0301.i

Citation: Shuja MN, Riaz H, Jamal M, Imran M. Kanamycin in cereal biotechnology: a screening or a selectable marker? *BioSci Rev.* 2020;2(3):01–05.

Journal QR

Article QR

Malik Nawaz Shuja

Indexing

A publication of the
Department of Life Sciences, School of Science
University of Management and Technology, Lahore, Pakistan

Letter

Kanamycin in Cereal Biotechnology: A Screening or a Selectable Marker?

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Article Info

Article history
Received: August 24th, 2020
Accepted: December 8th, 2020

Keywords
aminoglycoside,
cereals,
genetic transformation,
plasmid,
tissue culture

Abstract

Kanamycin is a widely used selection agent in dicot-plant genetic transformation systems. In monocots, however, it does not seem to be effective as it has no or minimal effect on the normal growth of non-transformed plants. Kanamycin was previously demonstrated to bleach the pigments of the non-transgenic plants. This may yield the idea that kanamycin can be used as an effective screening marker rather than a selectable marker in monocots.

Kanamycin, an aminoglycoside antibiotic, was first isolated in Japan in 1957. It was synthesized by the soil-borne actinomycete known as *Streptomyces kanamyceticus.* Kanamycin is a trisaccharide composed of a deoxystreptamine and two glucosamine units identified as 3-D-glucosamine-2-deoxystreptamine-6-o-glucosamine. A tetrasaccharide antibiotic neomycin is a related aminoglycoside with chemical properties very similar to kanamycin [1]. Neomycin is synthesized by another actinomycete known as *Streptomyces fragdiae*[2]. Other similar molecules are gentamicin (also known as Geneticin or G418), paromomycin, and hygromycin.

Kanamycin is toxic to plants, animals and fungi [2]. The active aminoglycoside antibiotics specifically bind to the ribosomal 30S subunit, thereby blocking the formation of initiation complexes and leading to the inhibition of protein synthesis. These antibiotics if used on plant cells affect mitochondria and chloroplast by impairing protein synthesis. Mitochondria and chloroplasts have ribosomes similar to those found in the bacteria and are, therefore, susceptible to aminoglycoside antibiotics. The antibiotics, if present, block chlorophyll synthesis (bleaching and chlorosis) and tissue growth [3, 4].

A kanamycin scavenging gene *nptII* (or *neo*), expressed by *Escherichia coli* transposon *Tn5*, encodes neomycin phosphotransferase II enzyme – also known as aminoglycoside 3'-phosphotransferase II [5, 6, 7]. This enzyme transfers the γ-phosphate group of

ATP to the 3'-hydroxyl group of the amino-hexose residue of the aminoglycoside antibiotics that results in detoxification. This ATP-dependent phosphorylation allows protein synthesis, thereby resisting the specific binding of antibiotics to ribosomes. To sum up, the kanamycin A, B, and C, neomycin, paromomycin, and geneticin – aminoglycoside antibiotics containing 3'-OH group – are therefore substrates of NPTII [8].

Kanamycin is among the most widely used selection markers in plant genetic transformation protocols. In a genetic transformation setting, kanamycin is supplemented to the growth medium in a concentration that may inhibit untransformed cells from regeneration. The exact mechanism of the transport and movement of aminoglycoside in plant tissue is not yet known. Specifically, kanamycin does not seem to be mobile in the vascular tissue; rather, it seemingly diffuse through the plant tissue via intercellular spaces. The diffusion may occur over short distances [9] suggesting that in large explants the antibiotic may not reach distal portions.

In plant genetic transformations, screening / selection (selection of marker) of the transgene is critical. In dicotyledonous plants, the use of kanamycin has proved to be very effective as a selection agent. However, monocots were found insensitive to the relatively high levels of kanamycin, thus allowing the regeneration of untransformed plant cells on media supplemented with kanamycin [10, 11, 12]. Even a high concentration of 100 µg/ml kanamycin could not restrict the growth of approximately 70% of untransformed rice calli [12]. Specifically, the protoplasts derived from the suspension culture of *Lolium perenne* were able to divide even in the presence of 800 µg/ml concentration of kanamycin [10]. The reason might be the presence of endogenous resistance rather than the transgene selection advantage. Collectively, these results indicate that in cereals kanamycin could not be used as a selection marker that could restrict / stop the growth of untransformed calli / cells. On the contrary, there are several reports of success with the use of kanamycin for the transformation of rice [13], wheat [14], and maize [15, 16].

In wheat and barley, kanamycin affects the chlorophyll synthesis, thereby bleaching the green pigment [17, 18]. Also, the non-transformed callus on kanamycin supplemented media regenerated as bleached (albino / white) plantlets [19]. Furthermore, no growth retardation or any other symptoms of the antibiotic except the green pigment bleaching were observed in wheat (Figure 1). Our analysis, therefore, suggests that kanamycin may better and efficiently be used as a screening marker rather than as a selectable marker.

Figure 1. Bobwhite wheat regenerated from the callus and screened on media supplemented kanamycin antibiotic.

A) White shoots emerged from the non-transformed calli on shoot induction media B) Shoots grown on root induction media (Left bottle: non-transgenic plant turned white on kanamycin, Right bottle: transgenic plant on kanamycin antibiotic).

The pictures were adopted from Ali [20].

References

[1] Kors FT. *Antibiotics: mode of action, spectrum, resistance, solubility,*

sterilization, stability. Hareelm: Duchefa:1993;1-30.

[2] Nap JP, Bijvoet J, Stiekema WJ. Biosafety of kanamycin-resistant transgenic plants." *Transgenic Res.* 1992;1(6):239-49.

[3] Benveniste RA, Davies J. Mechanisms of antibiotic resistance in bacteria. *Annu Rev Biochem*. 1973; 42:471-506. 10.1146/annurev.bi.42.070173.002351

[4] Brasileiro AC. Neomicina Fosfotransferase II (NPT II). *Manual de transformação genética de plantas*. Embrapa-Cenargen, Brasília;1998:143-54.

[5] Bevan MW, Flavell RB, Chilton MD. A chimaeric antibiotic resistance gene as a selectable marker for plant cell transformation. *Biotechnol*. 1992; 24:367-370.

[6] Fraley RT, Rogers SG, Horsch RB, et al. Expression of bacterial genes in plant cells. Proc. *Natl. Acad. Sci*. 1983;80(15):4803-7. https://doi.org/10.1073/pnas.80.15.4803

[7] Herrera-Estrella L, De Block M, Messens EH, et al. Chimeric genes as dominant selectable markers in plant cells. *EMBO J.* 983;2(6):987-995. https://doi.org/10.1002/j.1460-2075.1983.tb01532.x

[8] Norelli JL, Aldwinckle HS. The role of aminoglycoside antibiotics in the regeneration and selection of neomycin phosphotransferase-transgenic apple tissue. *J Am Soc Hortic Sci.* 1993;118(2):311-316. https://doi.org/10.21273/JASHS.118.2.311

[9] Weide R, Koornneef M, Zabel P. A simple, nondestructive spraying assay for the detection of an active kanamycin resistance gene in transgenic tomato plants. *Theor Appl Genet*.1989;78(2):169-172.

[10] Potrykus I, Saul MW, Petruska J, et al. Direct gene transfer to cells of a graminaceous monocot. *Mol Gen Genet*. 1985;199(2):183-188.

[11] Hauptmann RM, Vasil V, Ozias-Akins P, et al. Evaluation of selectable markers for obtaining stable transformants in the Gramineae. *Plant physiol*. 1988;86(2):602-606. DOI: https://doi.org/10.1104/pp.86.2.602

[12] Dekeyser R, Claes B, Marichal M, et al. Evaluation of selectable markers for rice transformation. *Plant Physiol*. 1989 ;90(1):217-23.

[13] Zhang HM, Yang H, Rech EL, et al. Transgenic rice plants produced by electroporation-mediated plasmid uptake into protoplasts. *Plant Cell Rep*. 1988;7(6):379-84.

[14] He Y, Jones HD, Chen S, et al. Agrobacterium-mediated transformation of durum wheat (Triticum turgidum L. var. durum cv Stewart) with improved efficiency. *J Exp Bot*. 2010;61(6):1567-1581. https://doi.org/10.1093/jxb/erq035

[15] Assem SK, Hussein EH, Hussein HA, et al. Genetic transformation of the Nicotiana protein kinase (NPK1) gene confers osmotic tolerance in Egyptian maize. *Aust J Basic & Appl Sci*. 2009;3(2):828-835.

[16] Omirulleh S, Ábrahám M, Golovkin M. Activity of a chimeric promoter with the doubled CaMV 35S enhancer element in protoplast-derived cells and transgenic plants in maize. *Plant Mol Biol.* 1993;21(3):415-28.

[17] Peters NR, Ackerman S, Davis EA. A modular vector for Agrobacterium mediated transformation of wheat.

Plant Mol Biol Rep. 1999;17(4):323-331.

[18] Öz MT, Eyidoğan F, Yücel M, et al. Optimized selection and regeneration conditions for Agrobacterium-mediated transformation of chickpea cotyledonary nodes. *Pak J Bot*. 2009;41(4):2043-54.

[19] Ali M, Tahir M, Hameed S.Genetic transformation of an elite commercial wheat variety through Agrobacterium tumefaciens. Int J. Biosci. 2016;8(1):17-24.

[20] Ali M. *Engineering resistance against barley yellow dwarf virus (BYDV) by expression of the virus derived gene(s) in a model plant.* PhD Dissertation. Atta-ur-Rahman School of Applied Biosciences. National University of Sciences and Technology, Islamabad, Pakistan; 2017.