Journal of Biotechnology and Bioengineering
Volume 3, Issue 2, 2019, PP 1-7
ISSN 2637-5362

A Comparative Study of the Effects of amino glycoside and ß-Lactam Antibiotics on Callus and Shoot Induction using Explants Infected with Agro bacterium

Phetole, Mangena[1*], Martha Chabalala and Andries Thangwana[2]

[1]Department of Biodiversity, School of Molecular and Life Sciences, Faculty of Science and Agriculture, University of Limpopo, Private Bag X1106, Sovenga, 0727, Limpopo Province, Polokwane, South Africa

[2]Irrigation and Climate Control Department, 63 Tarlton 1749, Flamingo Horticulture (South Africa)

***Corresponding Author:** Phetole, Mangena , Department of Biodiversity, School of Molecular and Life Sciences, Faculty of Science and Agriculture, University of Limpopo, Private Bag X1106, Sovenga, 0727, Limpopo Province, Polokwane, South Africa, Email: phetole.mangena@ul.ac.za*

ABSTRACT

Agrobacterium-mediated transformation offers great opportunities to induce desired characteristics in various plants for breeding purposes. But, this technique remains aloof for soybean improvement due to setbacks caused by inefficient regeneration systems. The dual effect of antibiotics (aminoglycoside and ß-lactam antibiotics) and Agrobacterium on callus initiation and shoot induction using cotyledonary node explants were investigated. Seeds of soybean cultivar LS677 and LS678 were used in this study. The seeds were disinfected using chlorine gas and germinated on MS mediumcontaining1.78 µM 6-benzyladenine (6-BA) to develop seedlings used as source of double cotyledonary-node explants. The prepared explants were co-cultivated with Agrobacterium, and then evaluated for callus and multiple shoot induction on MS media supplemented with the different types and concentrations of antibiotics. According to the study, explants sub cultured on MS medium containing aminoglycosides induced a high number of shoots than ß-lactamantibiotics which were effective for callus induction. This study suggests that aminoglycoside antibiotics inhibited callogene sis, mean while, ß-lactams exerted negative pressure on shoot proliferation required for efficient Agrobacterium-mediated transformation.

Keywords: *Agrobacterium; aminoglycoside antibiotics; cotyledonary explants; callus induction; Glycine max; shoot induction; ß-lactam antibiotics*

INTRODUCTION

Soybean has recently attracted global attention as an important oilseed crop but, it is currently facing growth challenges due to its high susceptibility to environmental stress [1, 2, and 3]. Poor seed germination, scanty vegetative growth, decreased yields which remains fundamentally and specifically caused by abiotic stress, destructive pests and fungal infections limit soybean cultivation worldwide [2,3].

As such, the unavailability of genetically improved stress tolerant varieties, designed to fully adapt to adverse agro climatic conditions pose a major challenge [4].

Plant genetic transformation via Agro bacterium tumefaciens coupled with plant tissue culture (PTC) still offer a useful tool to potentially obtain high yielding, healthy and improved soybean plants [5]. This technique as successfully produced elite genotypes than conventional breeding using natural and simple means to achieve genomic changes in plants [6]. Although, the use of A tumefaciens is considered a cheaper method of choice for many laboratories, in vitro regeneration of transgenic plants via this tool remains highly recalcitrant, genotype-specific and inefficient [6,7].

The optimization of tissue culture conditions, e.g. the use of antibiotics, which play a critical role in controlling and eliminating hurdles faced during co-cultivation of explants with Agro bacterium, is deemed necessary [8]. Antibiotics also serve as a selection regime for isolating non-transformed plants from transgenic ones [9]. However they remain ineffectively used and neglected in many transformation cultures. Several reports have also demonstrated their negative effects during the establishment of callus and shoot cultures [4-10]. Generally, the

appropriate choice of suppression and selective agents is vital to avoid excessive use of these compounds.

Therefore, thorough attention must be afforded to culture optimization in order to accelerate genetic manipulation of recalcitrant soybean genotypes [8, 11].

The modification of soybean in vitro transformation protocol could provide insights that thoroughly explain why genetic manipulation in soybean continues being inconsistent.

Furthermore, the relationship between recalcitrance and poor in vitro soybean regeneration to genetic transformation may be well understood.

Thus, the main objective of this study was to comparatively evaluate the effect that Agro bacterium, amino glycosides and ß-lactam antibiotics have on callus and shoot induction for subsequent Agro bacterium-mediated genetic transformation in soybean.

Materials and Methods

Plant Material and Explant Preparation

Seeds of two soybean (Glycine max L.) cultivars; LS677 and LS678 were used in this study. The seeds were surface sterilised over-night using chlorine gas for 16-hours [8]. Decontaminated seeds were then inoculated on MS basal culture medium supplemented with1.78 µM 6-benzyladenine (6-BA).

For germination Seed cultures were then incubated in a growth room, at 50−60 µmolm-2s-1 light intensity, 24±2°C temperature and under 16-hour photoperiod for 10-days.

The cotyledonary node explants from 10-day old seedlings, were prepared by transversely cutting-off the hypocotyls, 5 mm beneath the cotyledons, and removing the epicotyls from the base [12].

These explants were used for the establishment of callus and shoot induction cultures.

Bacterial Culture

Agro bacterium tumefaciens culture strain EHA101, harbouring a binary plasmid vector pTF101.1 was used in this study. The bacterium was reinitiated on yeast extract peptone (YEP) medium containing 3.01 µM spectinomyc in and 1.03 µM kanamycin as selective agents. Then Agro bacterium was collected by pelleting at 3,500 rpm for 10-min at 20°C and the pellet resuspended in Gamborg's B5 liquid infection medium [5,13].

Infection and Co-Cultivation of Cotyledonary Explants

Cotyledonary node explants were infected with Agrobacterium by completely immersing them in liquid infection medium containing bacterial cells, and then in cubated on an orbital-shaker for 20-min, at room temperature [5]. After 20-min, the infected explants were placed on Gamborg's B5 co-cultivation medium over-laid with sterile What man no.1 (90 mm) filter papers [5].

The plates were wrapped with a parafilm and incubated for 4-days in a tissue culture growth room. A total of 120cotyledonary-nodes were prepared per cultivar, for 8 replicates containing 15-explants each. Each petri plate (90 x 15 mm) contained 20 explants with their adaxial surfaces touching the filter paper.

Effect of Agro bacterium on Callus

After co-cultivation, the infected explants were rinsed several times with sterile distilled water to remove excess Agro bacterium. The washed explants were then cultured in MS culture medium supplemented with different concentrations of antibiotics and PGRs for callus induction (Table 1). The amounts of growth regulators used to test for callus formation were predetermined during a preliminary study. For cell proliferation and callus initiation; cultures were maintained in a tissue culture growth room under conditions as used above for three to four weeks.

Table1. *Type and concentration of plant hormone and antibiotics used for callus and shoot induction medium*

MS culture medium for callus induction				
	MS-1	**MS-2**	**MS-3**	**Ctrl**
Hygromycin	9.47	3.79	--	--
Tetracycline	113	2.25	--	--
Rifampicin	6.08	1.22	--	--
Cefotaxime	--	--	3.45	--
Vancomycin	--	--	2.20	--
Kinetin	3.25	3.25	3.25	3.25
IBA	1.33	1.33	1.33	1.33

NAA	6.45	6.45	6.45	6.45
MS culture medium for shoot induction				
	MS-1	**MS-2**	**MS-3**	**Ctrl**
Hygromycin	9.47	3.79	--	--
Tetracycline	113	2.25	--	--
Rifampicin	6.08	1.22	--	--
Cefotaxime	--	--	3.45	--
Vancomycin	--	--	2.20	--
6-BA	3.25	3.25	3.25	3.25

MS- Murashige and Skoog (1-3) basal culture medium; IBA- indole-butyric acid; 6-BA- 6-benzyladenine and NAA- naphthalene-3-acetic acid

Multiple Shoot Regeneration

For multiple shoot induction, explants co-cultured with Agro bacterium were placed with their adaxial surfaces firmly in contact with MS medium supplemented with 8.88 µM 6-BA (Table1). Cotyledonary node explants un-infected with the bacterium were used as controls. All the cultures were maintained at 24±2°C under 16-h photoperiod with photo-synthetic photon flux density of 50-60 µmolm-2s-1 provided by cool white fluorescent lights.

Effect of Antibiotics on Callus and Shoot Induction

To study the effect of antibiotics on callus and shoot induction, cotyledonary explants were cultured with their adaxial surfaces in firm contact with the medium. The explants were cultured for 3-4 weeks on MS medium containing the different combinations of PGRs and antibiotics as indicated on the table (1). Two categories of broad spectrum plant tissue culture tested antibiotics were used in this study, specifically the amino glycosides and ß-lactam antibiotics (Table 1).

In Vitroelongation and Rooting

After adventitious shoot formation, induced microshoots were excised-off the explants and sub cultured for elongation, followed by in-vitrorooting on MS medium without plant growth regulators.

All cultures were maintained in a tissue culture growth room under conditions similar to those used for seed germination, callus culture and shoot induction.

All regenerated plantlets, including shoots derived from morphogenic callus were acclimatised in a growth room before transfer into a glasshouse under natural conditions [12].

Statistical Data Analysis

Percentage seed germination, callus proliferation, morphogenesis and multiple shoot induction rates were monitored as growth parameters evaluated for every media composition and soybean cultivar. Results on callus formation found in tables represent the mean percentage ± standard error of 15 replicates containing two explants each and the experiment was repeated three times. All data were analyzed using the analysis of variance in IBM SPSS Statistics 24

Results

Effect of Antibiotics on Callus and Shoot Induction

Callus and shoot induction on MS medium without antibiotics exhibited the highest rates of callus proliferation and shoot multiplication compared to media containing antibiotics (Table 2 and 3).

This was achievable using explants successfully developed from seeds germinated on medium supplemented with 1.78 µM 6-BA, with over all germination percentage of 80.5 and 92.3%in cultivar LS678 and LS677, respectively.

The morphology of developed seedlings displayed highly reduced epicotyls, hypocotyls, primary roots without lateral roots and broadly opens dicotyledons.

Little callus often with brownish compacted cells were obtained on MS media supplemented with antibiotics (Fig.1A, B). Generally, callus obtained from medium with aminoglycoside and ß-lactam antibiotics appeared oxidised, consisting of dark brown cells mainly at the hypocotyl bases of the explants.

Coty-nodes sub cultured on medium supplemented with amino glycosides produced very little amounts of light green callus to predominantly white microcalli (Fig. 1 B) that

turned brown and died after just 4-weeks of incubation. As a result, significantly lower amounts of callus (33.5 and 42.5%) were obtained in this culture compared to 55.3 and 60.5% on media containing ß-lactams in LS677 and LS678, respectively (Table 2 and 3).

Table2. *Response of cotyledonary explants sub cultured on MS medium containing amino glycoside antibiotics for callus and shoot induction*

Plant cultivar	Callus formation (%)	Morphogenic response (%)	Mean shoots ± SE	Ave shoot length (cm)	Ave root length (cm)	Coefficient of variance
LS677	33.5±5.2^{d}	39.0^{a}	5.4±2.9^{d}	4.4^{cb}	4.2^{b}	18.1
LS678	42.5±4.4^{c}	17.5^{b}	5.1±1.7^{c}	3.3^{d}	2.6^{d}	18.3
Ctrl- 1	84.3±1.7^{a}	Nil	14.1±3.9^{a}	4.7^{a}	4.4^{a}	20.0
Ctrl- 2	80.5±2.9^{b}	Nil	13.3±2.8^{b}	4.4^{bc}	3.9^{c}	21.4

Ctrl- 1 & 2 refers to control on cultivar LS677 and LS678, respectively. Mean values within columns followed by superscript letters significantly differ from each other at 95% confidence level according to ANOVA

A high number of explants from LS678 induced fairly large amounts of callus than in LS677, which directly initiated shoot buds. As indicated on Table 2 and 3, LS677 gave the highest overall morphogenic callus response (39%) than LS678 with the highest overall mean percentage of 23.6%.

However, explants inoculated on control media efficiently formed light-green to slightly yellow friable callus as indicated in Fig. 1 (C).

Cells induced in this medium grew faster and were in significantly higher amounts in all soybean cultivars used. Profuse shoot multiplication was also observed in this study.

Shoots directly induced from cotyledonary explants and those recovered from caulogenic effects were maintained.

All shoots developed from the junction meristems, within two weeks on a medium containing 8.88 µM 6-BA (Fig. 1 D-F).

Culture medium without antibiotics induced higher number of shoots (Fig. 1 D) than medium amended with antibiotics (Fig. 1 E, F).

In addition, the amino glycosides induced high mean number of shoots, especially in cultivar LS677 than LS678 (Table 2), and compared to ß-lactam antibiotics (Table 3).

Table3. *Response of cotyledonary explants sub cultured on MS medium containing ß-lactam antibiotics on callus and shoot induction*

Plant genotypes	Callus formation (%)	Morphogenic response (%)	Mean shoots ± SE	Ave shoot length (cm)	Ave root length (cm)	Coefficient of variance
LS677	55.3±0.3^{d}	10.5^{b}	4.4±0.2^{d}	3.9^{d}	3.2^{d}	24.3
LS678	60.5±0.1^{c}	23.6^{a}	3.6±0.3^{c}	5.5^{a}	5.2^{a}	12.0
Ctrl-1	84.3±1.7^{a}	Nil	14.1±3.9^{a}	4.7^{b}	4.4^{b}	20.0
Ctrl-2	80.5±2.9^{b}	Nil	13.3±2.8^{b}	4.4^{c}	3.9^{c}	21.4

Ctrl-1& 2 refers to control on cultivar LS677 LS678, respectively. Mean values within columns followed by superscript letters significantly different from each other at 95% confidence level according to ANOVA

Effect of Agro bacterium on Callus and Shoot Induction

In cultures where cotyledonary explants showed capability to form callus and clusters of multiple shoot buds, the percentage of callus and induced shoots still significantly declined with the infection of explants.

With Agro bacterium there was no efficient callus proliferation observed on explants infected with Agro bacterium.

Suggesting that the production of callus, as well as regenerative calli cells were greatly affected by the infection Table4.

Similarly, cotyledonary node explants infected and co-cultured with Agro bacterium failed to stimulate profuse growth of multiple adventitious shoots as seen above. Under developing buds and few highly reduced shoots were observed on more than 70% explants infected with Agro bacterium (Fig. 1 G, H).

But, that did not translate into vigorous bud and shoot growth. A few intact and stunted adventitious shoots were observed on some explants, ranging between 0.4-2.9 shoots per explants (Fig. 1 H).

When uninfected explants were cultured on the medium without antibiotics, 13 to 14 mean

number of shoots was obtained per explants as presented on the tables. Which were higher than shoots induced on infected explants or media supplemented with antibiotics (Table 2, 3, 4).

Table4. *Response of cotyledonary explants co-cultured with A. tumefaciens transformed with the binary vector pTF101.1, containing bar gene for herbicide resistance on callus and shoot induction*

Plant genotypes	Callus formation (%)	Morphogenic response (%)	Mean shoots ± SE	Ave shoot length (cm)	Ave root length (cm)	Coefficient of variance
LS677	Nil	Nil	2.7 ± 0.2^{d}	Nil	Nil	35.8
LS678	Nil	Nil	2.9 ± 0.1^{c}	Nil	Nil	28.5
Ctrl-1	84.3 ± 1.7^{a}	Nil	14.1 ± 3.0^{a}	4.7^{b}	4.4^{b}	20.0
Ctrl-2	80.5 ± 2.9^{b}	Nil	13.3 ± 2.8^{b}	4.4^{c}	3.9^{c}	21.4

Ctrl-1& 2 refers to control on cultivar LS677 and LS678, respectively. Mean values within columns followed by superscript letters significantly different from each other at 95% confidence level according to ANOVA.

In Vitro Elongation, Rooting and Hardening

A high number of in-vitro elongated, rooted and acclimatized shoots was achieved from the control. Better shoot elongation of about 3-5 cm in length and rooting (3-4 cm) were achieved on MS medium without PGRs (Fig1I). In comparison, all shoots and shoot clumps formed on medium supplemented with antibiotics exhibited difficulties for elongation and rooting. On average, the frequency of shoot elongation and rooting ranged between 20-40% on media containing antibiotics (for both amino glycosides and ß-lactams) and 80-95% in shoots sub cultured on medium without antibiotics (Table 2 and 3). A large number of shoots that were successfully established from the media with antibiotics did not efficiently produce adventitious roots.

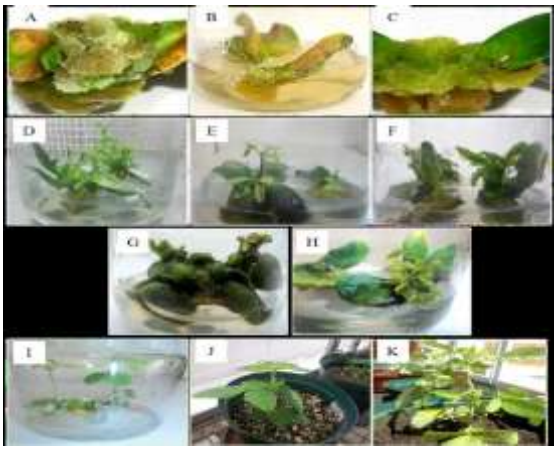

Figure1. *Effect of antibiotics and Agrobacterium on callus and shoot induction using cotyledonary node explants. Example of callus proliferation on medium supplemented with ß-lactams (A), aminoglycosides (B) and without antibiotics (C). Shoot formation on medium without antibiotics (D), with aminoglycosides (E) and ß-lactams (F). Response of Agrobacterium co-cultured explants for shoot induction on MS medium with ß-lactam (G) and aminoglycoside (H) antibiotics. In-vitro elongation and rooting of survived shoots (I), plantlets acclimatisation (J), and further growth and development of regenerated plants in the glasshouse (K).*

Consequently, fewer shoots developed from explants infected with Agro bacterium did not survive until rooting. Rooting occurred within two weeks of culture, with some shoots initially displaying masses of brown and white compact callus cells on the cut bases of shoot stems, particularly in cultivar LS677.Furthermore, there was no acclimatization step for shoots induced from explants infected with Agro bacterium except for micro shoots obtained on MS culture media with antibiotics (Fig. 1 I and K). The inefficiency of shoot regeneration in the presence of antibiotics or infection of explants led to the complete failure of plantlets to reach acclimatization stages and further growth of plants.

Discussion

The results obtained in this study clearly indicated that, callus and shoot formation was influenced by the Agro bacterium, amino glycosides and ß-lactam antibiotics used. The selected PGRs were also suitable for efficient establishment of in vitro callus and shoot induction cultures. The suitability of explants and their efficiencies were previously reported

[12]. This was also supported by the use of cotyledonary explants derived from 3-day old immature seedlings germinated in MSB5 medium preconditioned with 2.0 mg L-1 6-BA.In Vigna radiata L. Wikzek, cultivar ML-267 [15] in nature, callus formation occurs as a result of wounding, due to the stimulation of endogenously expressed plant hormones; auxins and cytokinins [1]. However, the transfer of explants onto selected modified medium induced cell division and proliferation of tissues into callus. The variations observed in callus characteristics were mainly due to the composition of the media, as reflected by the variance co efficiencies. Similar observations were made in the evaluation of kanamycin on callus initiation using cotton hypocotyls and cotyledons to generate somatic embryos [16]. The results showed that while, different types of antibiotics were used, ß-lactams stimulated a better callus response in all cultures. Enhanced callogenes is using two hybrid aspens; Populus tremuloides x P. tremula and P. x canescense x P. gradidentata was also reported [17]. As far as literature is concerned, this is the first study that evaluated the effectiveness of amino glycosides on shoot induction during Agro bacterium-mediated genetic transformation given a wide range of reports in common bean, cowpea, soybean and pigeon pea [18-21].The results indicated that tissue senescence may be reduced when explants are co-cultured with a tumefaciens in the presence of amino glycosides such limitations were also reported in various studies indicating.

That high frequency of oxidative stress occurred in medium containing ß-lactam antibiotics (at concentrations above 500 mgL-1) [11, 17, and 22]. The results suppression of shoots initiation was instigated by both Agro bacterium and antibiotics in the medium, and such shoot induction dynamics were further reported [4, 6]. Agro bacterium overgrowth of latent bacterial cells still causes contaminations, especially on media with ß-lactam antibiotics.

Many studies emphasised that GA3 played a significant role in the efficient elongation of in-vitro developed shoots [5, 7, 23, 24], including abnormal promotive effects in other plant species [25, 26]. Elongation and rooting of shoots using 1.0 mg L-1 GA3 in combination with 4.0 mg L-1 BAP was reported [23]. However, this study indicated that inhibition of shoot and bud elongation, as well as rooting was achieved using MS medium without PGRs. This was critical in minimizing sudden impairment and sensitivity to hardening conditions of in-vitro regenerated plants as a result of the changes in environmental conditions [27]. In vitro conditions may cause negative effects on the ex-vitro establishment of regenerated plants, particularly on their transfer out of tissue culture with high survival rates [28].

Conclusion

The lack of a highly efficient Agro bacterium-mediated transformation protocol continues to hinder the transformation progress. Results obtained in this study indicated that amino glycosides may be considered as an alternative, replacing ß-lactam antibiotics, for shoot induction but, not callus induction during in vitrogenetic manipulation of soybean.

References

[1] Taiz, L. and E. Zeiger, 2002. Plant Physiology, 3re Edn., Sinauer Associates, Sunderland, Massachusetts, USA, pp: 118-124.

[2] Mangena, P., 2018. Water stress: Morphological and anatomical changes in soybean (Glycine max L.) plants, In Andjelkovic V. (ed.), Plant, Abiotic Stress and Responses to Climate Change, Intech Open, London, UK, pp: 9-31.

[3] Mangena, P., and A. Thangwana, 2018. The role of plant gene transformation in oilseed crop improvement in Agriculture. J. Biotechnol Bioengineerin., 2: 19-25.

[4] El-Siddig, M.A., El-Hussein, A.A., Siddig, M.A.M., M.M.A. Elballa and M.M. Saker, 2009. Agro bacterium-mediated transformation and (Lycopersiconesculenum Mill.) plants cv. Castlerock. J Genet Engin Biotechnol., 7: 11-17.

[5] Paz, M.M., Martinez, J.C., Kalvig, A.B., T.M. Fonger and K. Wang, 2006. Improved cotyledonary-node method using an alternative explant derived from mature seed for efficient Agro bacterium-mediated soybean transformation. Plant Cell Rep., 25: 206-213.

[6] Yan, B., Reddy, M.S.S., G.B. Collins and R.D. Dinkins, 2000. Agro bacterium-mediated transformation of soybean [*Glycine max* (L.) Merrill] using immature zygotic cotyledon explants. Plant Cell Rep., 19: 1090-1097.

[7] Wang, G., and Y. Xu, 2008. Hypocotyl-based Agro bacterium-mediated transformation of soybean (Glycine max) and application for RNA interference. Plant Cell Rep., 27: 1177-1184.

[8] Grzebelus, E., and L. Skop, 2014. Effect of ß-lactam antibiotics on plant regeneration in carrot protoplast culture. In Vitro Cell Dev Bio Plant 50: 568−575.

[9] Yukawa, K., Kaku, T., Tanaka, H., Y. Koga-Ban and M. Fukuda, 2008. Enhanced soybean infection by the legume "super-virulent" Agrobacterium tumefaciens strain KAT23. Bioscience, Biotechnol and Biochem J., 72: 1809−1816.

[10] Opabode, J.T., 2006. *Agrobacterium*-mediated transformation of plants: Emerging factors that influence efficiency. Biotechnol Mol Biol Rev., 1(1): 12−20.

[11] Mangena, P., P.W. Mokwala and R.V. Nikolova, 2015. *In vitro* multiple shoot induction in soybean. Int J Agric Biol., 17: 838−842.

[12] Pierik, R.L.M., 1997. In Vitro Culture of Higher Plants, Martinus Mish off Publishers, UK.

[13] Paz, M.M., Shou, H., Guo, Z., Zhang, Z., A.K. Banergie and K. Wang, 2004. Assessment of conditions affecting *Agrobacterium*-mediated soybean transformation using the cotyledonary-node explants. Euphytica., 136: 167−179.

[14] Zhang, Z., Xing, A., P. Staswick and T.E. Clemente, 1999. The use of Glufosinate as a selective agent in Agrobacterium-mediated transformation of soybean. Plant Cell, Tiss Org Cult., 56: 37-46.

[15] Yadav, S.K., Krishna, M.G., Maheswari, M., M. Vanaina and B. Vekateswarlu, 2010. High frequency of multiple shoots and plant regeneration from cotyledonary nodal explants of mung bean [*Vigna radiata* (L.) Wilczek]. J Biochem Plant Biotechnol., 19: 267-270.

[16] Zhang, B.H., Liu, F., Liu, Z.H., H.M. Wang and C.B. Yao, 2001. Effects of kanamycin on tissue culture and somatic embryogenesis in cotton. Plant Growth Regulators. 33: 137-149.

[17] Bosela, M.J., 2009. Effects of ß-lactam antibiotics, auxins and cytokinins on shoot regeneration from callus cultures of two hybrid aspens, Populustremuloides x P. tremula and P. x canescens x P. gradidentata. Plant Cell Tiss Org Cult., 98: 249-261.

[18] Cru de Carvalho, M.H., Le, V.B., Zuily-Fodil, Y., A.T. Pham Thi and T.T.V. Kiem, 2000. Efficient whole plant regeneration of common bean (*Phaseolus vulgaris* L.) using thin-cell-layer culture and silver nitrate. *Plant Sci*., 159: 223-232.

[19] Soni, L.A., Usman, I.S., M.I. Faguji and S.M. Bugaje, 2015. Forwards efficient in vitro regeneration of cowpea (Vigna unguiculate L. Walp): A review. Bri Biotechnol J., 7: 174-182.

[20] Mangena, P., P.W. Mokwala and R.V. Nikolova, 2017. Challenges of *In Vitro* and *In Vivo Agrobacterium* -mediated Genetic Transformation in Soybean, In Kasai M. (ed.), Soybean- the Basis of Yield, Biomass and Productivity, Intech Open, London, UK.

[21] Asande, L.K., Indieka, A.S., Adero, M.O., S. Kiboi and N.O. Amugune, 2016. *In vitro* regeneration of pigeon pea using leaf explants. Afri Crop Sci., 24: 191-201.

[22] Tran, T.N., and N. Sanan-Mishra, 2015. Effect of antibiotics on callus regeneration during transformation of IR64 rice. Biotechnol Rep., 7: 143-149.

[23] Yashoda, B.E., Jadhav, P.V., Moharil, M.P., Dudhare, M.S., Kale, P., Nandanwar, R.S., S.S. Mane and R. Dani, 2013. Epigenesis through in-vitro regeneration in soybean amenable to genetic transformation. VEGETOS.26, 245-254.

[24] Verma, K., A. Rani and R. Saini, 2011. Efficient plant regeneration system from half seed explant of soybean (Glycine max (L.) Merrill). Soybean Res., 9: 63-71.

[25] Sugla, T., Purkayastha, J., Singh, S.K., S.K. Solleti and L. Sahoo, 2007. Micropropagation of Pongamiapinnata through enhanced axillary branching. In Vitro Cell Dev Biol-Plant., 43: 409-414.

[26] Purkayastha, J., Sugla, T., Solleti, S.K., A. Paul and L. Sahoo, 2008. Rapid in-vitro multiplication and plant regeneration from nodal explants of Andrographis paniculate: a valuable medicinal plant. In Vitro Cell Dev Biol-Plant., 43: 409-414.

[27] Pospisilova, J., Ticha, I., Kadlecek, P., D. Haisel and S. Plzakova, 1999. Acclimatisation of micropropagated plants to *ex vitro* conditions. *Biologia Plantarum*., 42: 481-497.

[28] Chandra, S., Bandopadhyay, R., V. Kumar and R Chandra, 2010. Acclimatisation of tissue cultured plantlets: from laboratory to land. *Biotechnol let*., 32: 1179-1205.

Citation: *Phetole, Mangena , Martha Chabalala and Andries Thangwana, "A Comparative Study of the Effects of amino glycoside and ß-Lactam Antibioticson Callus and Shoot Induction using Explants Infected with agro bacterium", Journal of Biotechnology and Bioengineering, 3(2), 2019, pp 1-7.*