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EFFECT OF DIFFERENT CULTURE MEDIA ON CALLUS CULTURES OF SOME GENOTYPE OF *MEDICAGO SATIVA*

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ABSTRACT

Eight genotypes of Lucerne as C-10, IG-1212, IL-75, A-3, LLC-9, LLC-3, Anand-2 and AL-95-12 obtained from Indian Grassland Fodder and Agroforestry Research Institute, Jhansi. This genotype observed the colour and texture of the callus from explants. The genotypic differences were found significant but the media differences were observed to be non-significant. The best callus quality in terms of callus colour was observed from both hypocotyl and epicotyls explant in genotype IG-1212 whereas the genotype AL-95-12 exhibited best callus colour quality from cotyledon explants. The best callus texture was observed in genotype Anand-2 and AL-95-12 from hypocotyls explants, in genotype C-10, IL-75 from epicotyl explant and in all the genotypes except LLC-3 in cotyledon explants. MS and SH medium supplemented with 2.0 mg/l 2,4-D, 1.0 mg/l NAA and 0.2 mg/l BAP in general showed the best callus quality from different explants.

Figure : 00 References : 18 Tables : 03

KEY WORDS : Callus colour , Callus culture, Explants, Genotype, Lucerne and texture.

Introduction

Medicago sativa belongs to family *Fabaceae* and is commonly known as alfalfa or Lucerne. Lucerne is one of the most important cultivated leguminous forage crops. Lucerne grown worldwide is used to feed livestock and also dehydrated to produce protein supplement to be fed to the animals of all classes. It has also been recognized for medicinal value to the sick animals. It is highly palatable and nutritious to the livestock of all classes. It contains about 15-20% Protein, 1.5% Calcium, 0.2% Phosphorous on the dry matter basis and serves as rich sources of vitamins A, B and D[2]. In India, it is the third most important forage crop after sorghum and berseem occupies one-million-hectare area of the cultivation and provides 60 to 130 tons of green fodder per hectare[7]. Biotechnology for the improvement of popular Indian alfalfa cultivars has not been attempted so far and also that alfalfa improvement in India had been attempted marginally. Alternatively, transgenic approach involves incorporation of specific and useful gene into alfalfa to improve forage quality, or to produce novel industrial/ pharmaceutical proteins. Ideally, forage alfalfa needs to possess more fermentable carbohydrates, proteins with balanced amino acid profile. Thus, there is a need for identifying genotypes amenable for *in-vitro* manipulation among the existing varieties so that they could be improved for fodder yield and quality traits and specific alien genes could be incorporated in their genome.

Material and Methods

The experimental material consisted of the following eight genotypes C-10, IG-1212, IL-75, A-3, LLC-9, LLC-3, Anand-2 and AL-95-12 of Lucerne obtained from Indian Grassland Fodder and Agroforestry Research Institute, Jhansi. Sterilization of glasswares and instruments was done in autoclave at 15psi (121ºC) for 20 minutes. The explants used for *in-vitro* culture, consisted of 5-7days old and hypocotyl, epicotyl and cotyledon derived from *in-vitro* germinated seeds. Sterilization of the explants and inoculation in the media done in a laminar air flow cabinet to aseptic conditions. The mature seeds were surface sterilized and germinated on MS basal medium. After required period (5-7 days), the hypocotyl, epicotyl and cotyledon were excised aseptically from the seedling and cut into small pieces of 3-5mm length. About 4-5 pieces were placed on the medium in each flask of 100ml. Preparation of stock solution of culture media were used[1,14,16]. The callus colour (Brown callus, Dark yellow callus, light yellow callus and Green callus) and callus texture (Watery, Granular and Friable) was recorded on a visual scale from 1 to 4. Callus induction and subsequent callus maintenance, all cultures were kept in dark at 25±2ºC. Callus was maintained by successive subcultures at a regular interval of 20-21 days on MS medium with 2,4-D@2mg/l, NAA@1mg/l and BA@0.2mg/l of medium. The statistical methods were followed[3]. The visual score given for callus

TABLE-1 : Effect of different media and explants on colour and texture.

Media	Hypocotyls		Epicotyl		Cotyledon		Mean	
	Colour	Texture	Colour	Texture	Colour	Texture	Colour	Texture
SH	3.0	2.0	3.5	2.0	4.0	2.0	3.5	2.0
MS	3.5	2.0	3.5	2.0	4.0	2.0	3.67	2.0
Blaydes	2.0	2.0	2.0	2.0	4.0	2.0	2.67	2.0
Mean	2.8	2.0	3.0	2.0	4.0	2.0	-	-
Kw	3.91	0.69	9.87	0.02	0.56	0.00	-	-

colour and texture were analyzed using a non-parametric Kruskal-Wallis statistic[8] since these data were recorded on ordinal scale.

Result

The mature seeds of LLC-3 genotype of Lucerne were germinated aseptically on MS basal medium. The first response of the seeds of Lucerne on any tissue culture medium was their germination and initial fast growth of the seedlings differentiating into various organs, prior to callus formation. Hence, the hypocotyls, epicotyls and cotyledons from seven days old seedlings were harvested, cut into small pieces of 3-5mm length and inoculation aseptically on these different basal media, namely SH, MS and Blaydes supplemented with 3.0mg/l 2,4-D and 0.5 mg/l kinetin for evaluating their performance for callus quality (callus colour and texture) response. Callus quality was recorded on a visual score scale (1 to 4).

On the basis of visual observation on these callus quality parameters, there was no overall difference in the callus texture among all the three media, but for callus colour, MS medium was found best (3.67) followed by SH medium (3.5). The mean performance of Blaydes medium was lowest for these parameters (2.67). the cotyledon explants exhibited best quality of callus in terms of callus colour (4.0) followed by epicotyls (3.0) and hypocotyl (2.8) explants (Table-1). However, highly significant differences were observed from epicotyls explant.

Calli were induced in eight genotype of Lucerne from three explants *viz.,* hypocotyls, epicotyls and cotyledon on various culture media. Callus response was recorded in terms of callus quality (colour and texture). The calli exhibited a range of colour, such as white, whitish yellow or yellowish white, light yellow, yellow, green and brown in combination with different types of texture, such as vitrious, granular, friable and nodular. Callus colour and texture were recorded on a visual score scale ranging from 1 to 4, separately.

Effect of different genotypes and media on callus colour from hypocotyls, epicotyls and cotyledon explants is presented (Table-2). Genotypic and media differences for colour of hypocotyl derived callus were significant. The genotype IG-1212 scored maximum (3.21) for callus colour. This score indicates that most of the callus induced in this genotype was light yellow and greenish white. The least score (2.21) for colour was recorded by genotype C-10. This indicates for deep and light-yellow callus. All other genotypes were comparable to both of these genotypes. The MS medium with 2.0 mg/l 2,4-D, 1.0 mg/l NAA and 0.2 mg/l BAP scored (3.56) for callus colour and least score (1.44) was exhibited by SH medium with 4.0 mg/l IAA and 1.0 mg/l kinetin. This least score indicates for brown and deep yellow callus on this medium.

In epicotyls explants, significant differences for callus colour were observed in different genotypes and media. All the genotypes and media recorded the visual score of more than 3 for callus colour. This suggested that most of the callus induced from this explant was light yellow, greenish and greenish white. Genotype IG-1212 (3.8) and SH medium with 2.0 mg/l 2,4-D, 1.0 mg/l NAA and 0.2 mg/l BAP (3.75) recorded the maximum score for callus colour. Least score for callus colour was recorded in genotype LLC-3 (3.1) and SH medium with mg/l NAA and 1.0 mg/l kinetin (3.25).

Genotypic and media difference were significant with respect to callus colour from cotyledon explants. Genotype AL-95-12 (2.93) exhibited maximum score for callus colour that was yellow to light yellow followed by C-10 (2.79), IG-1212 (2.5), IL-75 (2.5), LLC-9 (2.29), LLC-3 (2.57) and Anand-2 (2.57) were statistically at par with each other. Least score for callus colour was recorded in genotype A-3 (1.86) that was brown to dark yellow. MS medium with 2.0 mg/l 2,4-D, 1.0 mg/l NAA and 0.2 mg/

TABLE-2. Effect of genotypes and media on colour of callus from hypocotyl, epicotyl and cotyledon explants

Genotypes	Explant	SH+1.0 mg/l NAA+1.0 mg/l Kinetin	SH+2.0 mg/l NAA+1.0 mg/l Kinetin	SH+4.0 mg/l NAA+1.0 mg/l Kinetin	MS+2.0 mg/l 2, -4D+1.0 mg/l NAA+2.0 mg/l BAP	SH+2.0 mg/l 2, -4D+1.0 mg/l NAA+2.0 mg/l BAP	SH+2.0 mg/l IAA+1.0 mg/l kinetin	SH+4.0 mg/l IAA+1.0 mg/l kinetin	Mean
C-10	Hypocotyl	3.5	3.5	2.5	3.5	1.0	1.0	0.5	2.21(b)
	Epicotyl	3.0	3.0	4.0	3.0	4.0	-	-	3.4(ab)
	Cotyledon	3.0	3.5	3.5	4.0	3.5	1.0	1.0	2.79(ab)
IG-1212	Hypocotyl	3.5	2.5	3.0	3.5	3.0	3.5	3.5	3.21(a)
	Epicotyl	3.5	4.0	4.0	3.5	4.0	-	-	3.8(a)
	Cotyledon	2.0	3.5	3.0	3.5	3.5	1.0	1.0	2.5(abc)
IL-75	Hypocotyl	3.5	3.5	3.0	3.5	3.5	1.0	1.0	2.71(ab)
	Epicotyl	4.0	3.0	4.0	3.0	4.0	-	-	3.6(ab)
	Cotyledon	3.0	3.0	3.0	3.5	3.0	1.0	1.0	2.5(abc)
A-3	Hypocotyl	3.0	3.0	1.5	3.5	3.0	1.5	1.5	2.43(ab)
	Epicotyl	3.0	3.0	4.0	3.5	4.0	-	-	3.5(abc)
	Cotyledon	1.5	1.5	1.5	3.5	3.0	1.0	1.0	1.86(c)
LLC-9	Hypocotyl	2.0	3.5	2.5	3.5	3.5	2.0	2.0	2.71(ab)
	Epicotyl	3.5	3.5	3.5	3.5	3.5	-	-	3.5(abc)
	Cotyledon	2.5	2.5	2.0	2.5	3.5	1.5	1.5	2.29(ab)
LLC-3	Hypocotyl	3.5	3.5	2.5	4.0	3.5	1.0	1.0	2.71(ab)
	Epicotyl	3.0	3.0	3.0	3.5	3.0	-	-	3.1(c)
	Cotyledon	3.5	3.5	2.0	3.5	3.5	1.0	1.0	2.57(ab)
Anand-2	Hypocotyl	3.0	3.0	3.5	3.5	3.5	1.0	1.0	2.64(ab)
	Epicotyl	3.0	3.0	3.0	4.0	4.0	-	-	3.4(bc)
	Cotyledon	3.0	3.5	3.0	3.5	3.0	1.0	1.0	2.57(ab)
AL-95-12	Hypocotyl	2.0	2.0	4.0	3.5	4.0	1.0	1.0	2.5(a)
	Epicotyl	3.5	3.5	3.5	3.5	3.5	-	-	3.5(bc)
	Cotyledon	3.5	3.5	4.0	4.0	3.5	1.0	1.0	2.93(a)
Mean	Hypocotyl	3.0[b]	3.06[ab]	2.81[b]	3.56[a]	3.13[ab]	1.5[c]	1.44[c]	-
	Epicotyl	3.31[bc]	3.25[c]	3.62[ab]	3.5[abc]	3.75[a]	-	-	-
	Cotyledon	2.75[b]	3.06[ab]	2.75[b]	3.5[a]	3.31[ab]	1.06[c]	1.06[c]	-

Mean value with different alphabets in a row [] and column () are significant different as per Kruskal-Wallis non-parametric statistics.

TABLE-3. Effect of genotypes and media on texture of callus from hypocotyl, epicotyl and cotyledon explants

Genotypes	Explant	SH+1.0 mg/l NAA+1.0 mg/l Kinetin	SH+2.0 mg/l NAA+1.0 mg/l Kinetin	SH+4.0 mg/l NAA+1.0 mg/l Kinetin	MS+2.0 mg/l 2, -4D+1.0 mg/l NAA+2.0 mg/l BAP	SH+2.0 mg/l 2, -4D+1.0 mg/l NAA+2.0 mg/l BAP	SH+2.0 mg/l IAA+1.0 mg/l kinetin	SH+4.0 mg/l IAA+1.0 mg/l kinetin	Mean
C-10	Hypocotyl	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0(a)
	Epicotyl	2.0	2.0	2.0	2.0	2.0	-	-	2.0(a)
	Cotyledon	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0(ab)
IG-1212	Hypocotyl	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0(a)
	Epicotyl	2.0	1.0	1.0	1.0	1.0	-	-	1.2(b)
	Cotyledon	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0(a)
IL-75	Hypocotyl	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0(a)
	Epicotyl	2.0	2.0	2.0	2.0	2.0	-	-	2.0(a)
	Cotyledon	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0(a)
A-3	Hypocotyl	2.0	2.0	1.0	2.0	1.0	2.0	2.0	1.71(ab)
	Epicotyl	1.0	1.0	1.0	1.0	1.0	-	-	1.0(b)
	Cotyledon	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0(a)
LLC-9	Hypocotyl	2.0	2.0	1.0	2.0	2.0	1.0	1.0	1.57(b)
	Epicotyl	1.0	1.0	1.0	1.0	1.0	-	-	1.0(b)
	Cotyledon	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0(ab)
LLC-3	Hypocotyl	2.0	2.0	1.0	2.0	2.0	2.0	2.0	1.86(ab)
	Epicotyl	1.0	1.0	1.0	1.0	1.0	-	-	1.0(b)
	Cotyledon	2.0	2.0	1.0	2.0	2.0	1.0	1.0	1.57(b)
Anand-2	Hypocotyl	2.0	2.0	2.0	2.0	3.0	2.0	2.0	2.14(a)
	Epicotyl	1.0	1.0	1.0	1.0	1.0	-	-	1.0(b)
	Cotyledon	2.0	2.0	3.0	2.0	1.0	2.0	2.0	2.0(a)
AL-95-12	Hypocotyl	2.0	2.0	2.0	2.0	3.0	2.0	2.0	2.14(a)
	Epicotyl	1.0	1.0	1.0	1.0	1.0	-	-	1.0(b)
	Cotyledon	2.0	2.0	1.0	2.0	2.0	2.0	2.0	1.88(ab)
Mean	Hypocotyl	2.0[ab]	2.0[ab]	1.63[c]	2.0[ab]	2.13[a]	1.88[bc]	1.88[bc]	-
	Epicotyl	1.37[a]	1.25[a]	1.25[a]	1.25[a]	1.25[a]	-	-	-
	Cotyledon	2.0[a]	2.0[a]	1.88[a]	2.0[a]	1.88[a]	1.88[a]	1.88[a]	-

Mean value with different alphabets in a row [] and column () are significant different as per Kruskal-Wallis non-parametric statistics.

BAP recorded maximum score of callus colour. This score indicated for light yellow to greenish or whitish colour followed by SH medium (3.31). SH medium with 1.0 mg/l kinetin and 2.0 mg/l IAA or 4.0 mg/l IAA recorded least callus colour (1.06) score which was indicated for brown colour of callus.

Effect of different genotypes and media on texture of callus from hypocotyls, epicotyls and cotyledon explants (Table-3) show significant differences in different media and genotypes. Best callus texture was recorded in genotype Anand-2 (2.14) and AL-95-12 (2.14) which indicated that most of the callus was granular with little bit of friable callus. Poor (watery and granular) texture of callus was recorded in genotype LLC-9 (1.57) which was at par with A-3 (1.71) and LLC (1.57). Maximum score for callus texture was recorded in SH medium with 2.0 mg/l 2,4-D, 1.0 mg /l NAA and 0.2 mg/l BAP (2.13), which was comparable to SH medium with 1.0 mg/l kinetin and 1.0 mg/l NAA or 2.0 mg/l NAA and MS medium with 2.0 mg/l 2,4-D, 1.0 mg/l NAA and 0.2 mg/l BAP. SH medium with 4.0 mg/l NAA and 1.0 mg/l kinetin exhibited the least callus texture score that was comparable to that of SH medium with 1.0 mg/l kinetin and 2.0 mg/l IAA or 4.0 mg/l IAA.

The callus from epicotyls and cotyledon explants except for the genotype C-10 and IL-75 which recorded the maximum score (2.0) for callus texture, all other genotypes recorded 1.0 score for this trait. The callus from cotyledon explants of cotyledon for the genotype LLC-3 recorded the least score for callus texture, all other genotypes were comparable for this trait having granular type of callus.

Discussion

The basic foundation of plant tissue and cell culture system was laid down[6] by postulating the concept of "totipotency" in plant cells. Reversible totipotency of somatic plant cell is a specific and scientifically exciting phenomenon which is based on the general flexibility of the developmental program of plants[5].Cell suspension and eventually single cell cultures[17]. Cell and callus have stimulated the interest in this type of variations for their use in genetic improvement of different crops[18]. The increase in genetic variability through tissue cultures was first achieved in sugarcane followed by rice, wheat, lettuce and tomatoes[11]. *M. sativa* has been found amenable for *in-vitro* plant regeneration in number of studies[13]. Genotypes explants have been identified as important factors that influence *in-vitro* regenerating potential of Lucerne[12]. Workers[13] suggested that solidified MS medium with 2.0 mg/l 2,4-D and 1.5 mg/l kinetin was the best for callus induction in *M. sativa*. The SH medium gave the highest efficiencies in callus formation and plant regeneration[9]. Others[7]suggested that genotype background was most critical factor than either the medium or the nature of explants for callus induction and further regeneration. Some workers[15] found that the callus induction and regeneration responses were affected by explants sources.

Summary

The experiments on quality of the callus from different explants in eight genotypes, C-10, IG-1212, IL-75, A-3, LLC-9, LLC-3, Anand-2 and Al-95-12 of *M. sativa*. The effect of different basal media (SH, MS and Blaydes) from hypocotyl, epicotyl and cotyledon explants reeled that MS medium performed better than the other two media for callus colour and texture. Effect of explants on callus colour and texture also, the cotyledon explants were found best followed by epicotyl explants on MS medium. Genotypic effect was found prevalent for callus quality. Among all the eight genotypes, IG-1212 followed by AL-95-12 responded best for callus colour and texture.

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