In Vitro Technique for Heavy Metal, Cobalt Tolerance in Aloe Vera Callus

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Abstract

In vitro tissue culture application in plant biotechnology is an efficient plant propagation tool in arising plants resistant to diverse a biotic stresses such as drought and salinity. In this investigation, the aim on the in vitro breeding process applied for plants resistant to heavy metal (HM) stress. The experiment included the following two sequent stages: (i) callus cells initiation, some of which are soma clonal variation with new characteristics, (ii) susceptibility of the callus to HMs as selective factor during multiplication to select variants with enhanced HM-resistance. Aloe callus was grown on media complemented with 0.5, 1.0, 1.5 and 2.0 mg/l cobalt (Co) plus the control treatment. Cobalt accumulation level, mineral status, and callus growth were examined. Results indicate that the increase of Co concentration in medium had influenced callus growth giving the best growth 737 and 56.6 mg for fresh and dry weight respectively at 0.5 mg/l Co concentration, and that growth decreased with increase of Co concentration in medium. With concern to mineral status, cobalt had increased dramatically in callus cells with the increase of Co concentration treatment reaching 108.63ppm at 2.0 mg/l Cobalt, Fe ion concentration reached its highest level 1712.0ppm at 2.0mg/l Cobalt, Mn ions give the highest accumulation level 126.07 ppm at 1.0 mg/l cobalt. While potassium (K)and magnesium (Mg) decreased with increase of cobalt in callus medium recording their highest accumulation level 22244.0 and 492.47 respectively at plant control treatment. Na ions reached its highest level of accumulation 104.3 ppm at 0.5 mg/l cobalt concentration and decreased with the increase of cobalt concentration in the medium.

Key words: *Cobalt, tolerance, in vitro, Aloe*

Introduction

Soils generally include low levels of heavy metals, high levels can be hazardous to man, plants and animals. In attempt to preserve our environment, new techniques of remediation using chemical, physical and biological theories are being examined [(1)]. Phytoremediation is defined according to Cunningham et al. 1995 [(2)], as the utilization of plants to withdraw, embrace, or provide harmless environmental contaminants.

Phytoremediation offer many benefits compared to other remediation techniques: it is appropriate to a broad variety of contaminants; organic pollutants could be depraved to CO_2 and H_2O; it is cost-effective for vast areas having low to moderately contaminated soil surface [(3)].

Tissue culture is an effective mean that gives the solution to grow millions of cells under controlled conditions, moreover to obtain physiological facts about the action of plant cells under stress conditions [(4)]. Investigate and select at plant cell level established clones of plants with increased tolerance or defiance towards many environmental stresses like drought, heat, salt and heavy metals [(5)]. Plants are often sensitive to both high and low availability of some heavy metal ions as important micronutrients. Useful heavy metals at high level could hurt the soil environment that successively negatively influence soil fertility and plant growth [(6)].

Bakkaus et al. 2005 [(7)] mentioned the average Co concentration for plants among 0.1 and 10 μg g^{-1} dry weight and moreover detailed the beneficial part of Cobalt for the normal metabolic activity of plant at low

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concentration. Li et al. 2009 [8] studied toxicity of Cobalt on Hordeum, Lycopersicon and Brassica and reported that Cobalt has reduced growth and biomass in these plants shoots.

This study aimed to establish an in vitro procedure to assess the ability of Aloe vera tissue culture to accumulate heavy metals as an important step for biotechnology researches to select tolerant plants for HMs pollution.

Materials and Method

In vitro culture medium

Sterilized Explants (leaves) of Aloe vera L. were excised and cultured in universal tubes containing MS medium Murashige and Skoog, 1962 [9]. Different concentrations of the Auxin 2,4-D were tested(0.5, 1.0, 1.5,and 2.0 mg/l) which incubated in dark at a temperature 25 ± 1 °C for callus initiation [10].

Cobalt treatment

In vitro study was conducted using MS medium complemented with Cobalt, four different concentrations were tested: 0.5, 1.0, 1.5, and 2.0mg/l Cobalt, plus the control treatment, ten replicates were tested for each treatment. Cultures were maintained in a growth chamber at 25 ± 1 °C with a three-week subculture interval.

Callus growth evaluation

dry and fresh weight of callus were calculated, using three replicates for each treatment in the Auxin 2,4-D experiment, while regarding the heavy metal experiment using cobalt five replicates were used for each treatment.

Determination of Co essential and other trace elements content

Callus samples were previously dried in oven in order to determine the content of Fe, Ca, K, Cu, Mn, Na, Mg, and Co using Atomic Absorption Spectrometer (Solar Mb, thermo Fisher, USA).

Statistical Analysis

Biometrical data concerning the callus dry and fresh weight, and the content of cobalt and other chemical elements were subjected to Randomized complete design (CRD) analysis. The least significant difference L.S.D. test was used to study differences means between treatments at $P<0.05$ [11].

Results and Discussion

Callus initiation

Table 1 data reveals that dry weight of Aloe callus reached the highest weight (0.110 mg)at 1.0mg/l 2,4-D which differ significantly from the other 2,4-D concentrations treatment and from control treatment, while regarding the fresh weight of callus there was no significant difference between all 2,4-D concentrations treatment except from control.

Table 1: The effect of 2, 4 D concentration on fresh and dry weight of Aloe vera callus in mg

LSD 0.05	2,4 D mg/l					mean
	Cont.	0.5	1.0	1.5	2.0	
713.5	0.0 b	1241 a	1514 a	1640 a	1201 a	Fresh weight
0.042	0.0 c	0.101 b	0.110 a	0.103 b	0.102 b	Dry weight

Callus growth on cobalt supplemented media

Details in table 2 indicate a significant difference in Aloe callus fresh weight at 0.5mg/l cobalt concentration treatment recording 737mg which differ significantly from all except 2.0 mg/l cobalt treatments recording 657 mg, the same result was recorded for dry weight of Aloe callus at 0.5 mg/l cobalt concentration treatment giving 56.6 mg (table 2).

Table 2: Effect of cobalt concentrations on fresh and dry weight of Aloe vera callus in (mg)

LSD 0.05	Co concentration					mean
	Cont.	0.5	1.0	1.5	2.0	
122.9	396 b	737 a	509 b	515 b	657 a	Fresh weight
9.37	33.4 b	56.6 a	34.8 b	42.2 b	50.2 ab	Dry weight

Cobalt and other minerals concentration status in callus

Table 3 displays an increase with cobalt concentration in Aloe callus at 2.0 mg/l cobalt concentration treatment giving 108.63 ppm with a significant difference from the other cobalt concentration treatments and that cobalt accumulation decreased and reached its low level at callus and plant control treatments giving 10.27 and 0.0 ppm respectively. In regards to Fe ions, table 3 also shows that the highest accumulation of Fe ions was recorded at 2.0 mg/l cobalt concentration treatment registering 1712.0 ppm which differed significantly from control and the rest of cobalt concentration treatments. Table 3 also demonstrated that Na and Ca ions give the highest accumulation 104.3 and 40363.0 ppm respectively at 0.5 mg/l cobalt concentration treatment which differed significantly from the other treatments, while K and Mg ions decreased with the increase of cobalt concentration in medium reaching its highest levels at plant control treatment which differed significantly from the other treatments. Table 3 also resulted that Cu ions was at their lowest concentration level 0.363 ppm at 0.5 mg/l cobalt treatment.

Table 3: Effect of cobalt concentration on minerals accumulation of Aloe vera callus in (ppm)

LSD 0.05	Co concentration						mean
	Plant cont.	Callus cont.	0.5	1.0	1.5	2.0	
1.88	0.00 f	10.27 e	26.70 C	12.47 d	62.2 B	108.63 a	Co
24.86	38541.0 b	22029.0 e	40363.0 a	16551.3 f	24669.7 d	26416.0 c	Ca
5.397	492.47 a	12.75 f	233.64 B	113.80 d	91.27 e	143.77 C	Mg
16.51	22244.0 a	12336.0 f	19513.0 b	12956.7 d	12433.3 e	17913.3 c	K
0.4489	0.483 e	27.223 a	0.363 E	5.537 c	14.763 b	4.530 D	Cu
1.366	100.70 c	15.81 f	45.21 E	126.07 a	57.34 D	102.37 b	Mn
1.255	287.17 e	502.27 c	613.17 B	146.17 f	365.77 D	1712.0 A	Fe
1.276	45.54 e	61.21 d	104.3 A	20.58 f	70. 25 C	101.36 B	Na

Plants capture essential heavy metals like iron, zinc, manganese and copper from the soil due to concentration slope and selective uptakes of these metals [12]. The organic and inorganic manures are the agricultural origins of heavy metal contamination, irrigation water; liming, pesticides, and sewage discarding are the major cause of heavy metal release in the soil [13]. Chabukdhara et al. (2016) [14] revealed in a case study about heavy

metal in peri-urban and urban-industrial clusters in Ghaziabad, India, that waste water irrigation is behind the heavy load of HMs in agricultural soils. HMs ions strongly affected the activity of many enzymes and cellular metabolism; they also play an important role in protein, photosynthetic pigments, nucleic acids synthesis plus their role in the structural and functional integrity of cell membranes [15].

Cobalt plays an important role in growth and development of plants through regulating plant water utilization and reduce transpiration rate [16]. Gad and Hassan (2013) [17] conducted an experiment on tomato with Co application at 7.5 ppm, which improved growth, nutrients level and yield, although Arif et al. (2016) [3] reported that application of cobalt at 5µM on mung beans inhibited seedling growth and caused chlorosis in young leaves.

Hell and Stephan (2003) [18]; Thomine and Lanquar (2011) [19] stated that Fe is easily reduced and oxidized in many biochemical processes and it's an important cofactor in cytochrome. Cu ions had also an essential role in plant growth by engaging in many redox-active reactions, while Mn ions act in detoxification of ROS [20]. ROS could cause damage of photosynthetic pigments, plants generally response to ROS using antioxidant defense mechanism through synthesis of antioxidant enzymes such as superoxide dismutase, glutathione reductase and catalase that acts as scavengers of ROS [21].

Conclusion

Soil represents the most critical component gathering large amount of dangerous chemical pollutants from different sources per year. And according to Food and Agriculture Organization of UN report (FAO) in 2009, the world population will increase rapidly to reach about 9.6 billion till 2050. Thus, the future world challenge is to obscure the worlds hunger through prolonged agriculture and food production. Plants require some beneficial HMs in a limited quality. While, at high level these metals favor to create distinctive level of toxicity in plant that could inhibit plant growth, damage plant morphology and physiology. This create a need of much expand research on the mechanism of HMs uptake and translocation in relative to their impact on plant growth and development is obligatory to keep step with healthy agronomy production.

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