Callus is an unorganized mass of plant cells. Reliable callus induction and regeneration of viable plants is considered as a limiting step to the successful use of modern techniques in genetic improvement of the major crops (Murphy, 2003). In the present study, different PGR combinations were checked for in vitro callus induction in explants of Solanum tuberosum. Callus induction was found to be successful using different concentrations of 2,4-D alone and in combination with Kinetin but best combination was 2,4-D (13.59 μM) alone and of 2,4-D (9.06 μM) with Kinetin (1.16 μM). The auxin 2,4-D, by itself or in combination with cytokinins, has been widely used to enhance callus induction and maintenance. Moreover, many researchers observed 2,4-D as the best auxin for callus induction both in monocots and dicots (Chee, 1990 and Mamun et al., 1996). Role of 2,4-D in callus induction has been widely accepted and utilized for potato, tomato and many medicinal plants (Ashakiran et al., 2011; Ahmed et al., 2012; Lakshmi and Reddy, 2012; Mehta et al., 2012).

On the basis of regular observation it was concluded that the source of explant has a direct effect on callus induction. Results showed that leaf explant were more efficient for callus induction with 100% response as compared to internodes which gave only 50% response. This may be due to the presence of more meristematic activity in leaves as compared to internodes. This result supported the previous study by Haque et al. (2009) who found that callus length was affected by different explants and that the leaf explants produced significantly highest callus length in contrast to the shoot tip which produced least results in case of potato cv. Diamant. The interaction effect between explant and concentration of growth regulators were found to have significant differences on callus length in different researches. This result was also proved to be
significant by Dobranaszki et al. (1999) and Fomenko et al. (1998) who also observed significant effects of explants of potato on callus length. In the present study different concentrations of 2,4-D and Kinetin showed significant differences in callus growth and colour. Rate of callus induction increased with the increasing concentration of 2,4-D alone upto 13.59 µm and in the combination of 2,4-D and Kinetin upto 9.06 µm and 1.16 µm respectively. Further increase in concentration lead to decrease in callus growth and resulted in browning of callus. Callus initiation on cut ends of in vitro cultured explants of potato could be observed in all 2,4-D levels (Khalafalla et al., 2010). Similar findings were also reported by (Fieger et al., 2000, Jayasree et al., 2001 and Yasmin et al., 2003).

In Solanum different approaches so far have been adapted to obtain efficient in vitro regeneration system from petioles with intact leaflets (Shirley et al., 2001), leaves (Sarker & Mustafa, 2002; Anderson et al., 2003), tuber discs (Vasquez & Clarence, 2002), unripe zygotic embryos (Pretova and Dedicova, 1992), and from stem (Chang et al., 2002) after passing through callus phase. Osusky et al. (2005) reported regeneration of plants from leaf disc tissues during genetic modification of potato. Regeneration response in vitro is generally species and often genotype specific (Ritchie & Hodges, 1993). Therefore, regeneration conditions and characteristics may vary among genotypes and need to be determined prior to transformation (Hussain et al., 2005).

In vitro regeneration of potato is easily done from different explants on MS medium supplemented with different auxin and cytokinin for disease free good quality seeds and pathogen free planting materials (Rabbani et al., 2001, Zaman et al., 2001). Both callus induction and plant regeneration from explant require appropriate combinations and concentrations of plant growth regulators in the culture media.
(Ehsanpour et al., 2000). In the present research work, best results for shoot regeneration from callus of *Solanum tuberosum* was obtained by using a combination of 8.88 μM BAP and 1.00 μM GA₃ with significantly high average no. of shoots, shoot length and number of leaves per explant as compared to other combinations. BAP, Zeatin or Kinetin are known to help produce adventitious shoots. Martel *et al.* (1992) reported that both BAP and GA₃ at higher concentrations were necessary for shoot formation of potato. Shoot regeneration responses vary with the potato cultivar but in most cases cytokinin helps to enhance shoot production (Ghaffoor *et al*., 2003). Generally a low ratio of auxin to cytokinin is required for adventitious shoot development in case of potato (Anjum and Ali, 2004).

A decrease in all the parameters of shoot regeneration occurred after increase in the concentration of BAP after 8.88 μM. Similar effects of increasing concentration of BAP on shoot regeneration of potato cv. Asterix were observed by (Molla *et al*., 2011) who observed that the length of shoot increased with increasing BAP concentration up to 3 mg l⁻¹ and then decreased. Role of GA₃ in shoot elongation is well known and reported by many researchers. For rapid multiplication, addition of GA₃ to the MS media has been reported to improve growth and development of shoots. Farhatullah *et al.* (2007) also have reported that dosage of 0.248 mg l⁻¹ of GA₃ in the MS medium boosted all morphological characters in *in vitro* raised potato plantlets. Ullah *et al.* (2012) also have reported that GA₃ is involved in cell elongation and its addition in MS medium enhanced shoot growth in *in vitro* propagated plants of potato variety “Desiree”.

Direct regeneration system has an edge over regeneration after passing through callus phase to maintain the true-to-type nature of the regenerated plantlets and avoid somaclonal variation. Potato breeding programs can highly benefit from biotechnological
tools, which are capable of surpassing some limitations found by traditional plant breeding methods and open new avenues for crop improvement. The success of plant biotechnology relies on several factors which include an efficient tissue culture system for regeneration of plants from cultured cells and tissues (Khalafalla et al., 2010).

In the present study, attempts were made also made to induce direct regeneration of Solanum tuberosum. Explant used were nodes. Leaf discs and inter nodal tissues are the least responsive explants for direct regeneration. These explants underwent callus induction phase and then resulted in shoot regeneration indirectly in a study conducted by Hussain et al. (2005) on three potato cultivars viz., Cardinal, Altamash and Diamont. There are many advantages of taking nodal tissue as an explant, i.e., a large number of aseptic plants can be obtained quickly and easily, and plants produced may remain true to type.

Successful regeneration was obtained using hormonal combination of Zeatin, IAA and GA₃ in a concentration of 9.12 µM, 5.71 µM and 8.49µM, respectively. Role of Zeatin in regeneration has been reported by Wendt et al. (2001) who found that the internode explant of potato cultivar Macaca treated with Zeatin showed higher regeneration rate than those treated with BAP.

Genotype also play a vital role in shoot regeneration as well in transformation efficiency among potato varieties and other plants as reported in the literature (Phillip & Hampson 1995, Fitch et al., 2011). Creation of novel germplasm through techniques of tissue culture and gene transfer holds great potential for improving the quality, resistance to diseases and agronomic characters of potato (Ehsandar et al., 2013).
Roots were induced in microshoots using different concentrations of IBA, out of which 2.45 μM concentration emerged to be best with maximum average number of root (43.50) and a maximum average root length of 7.50 cm in full strength MS medium. IBA has been shown as a potent root inducer in many studies conducted on various tomato cultivars (Chaudhry et al., 2010; Khalafalla et al., 2010; Sakthivel and Manigandan, 2011).

Microtubers of Solanum tuberosum were obtained after incubating directly regenerated shoots at 16/8 h light/dark condition after 8-10 weeks. Microtubers obtained were green in colour. The green colour might be due to the presence of alkaloid solanin which is produced under light conditions. The edible part of the plant is the tuber, which is formed at the end of underground stems called stolon. Potato produced more protein and calories per unit area per unit time and per unit of water than any other major plant food. In vitro tubers can be produced throughout the year and thus holds benefit over conventional tubers (Hoque, 2010).

Drought stress is considered to be a moderate loss of water, which leads to stomatal closure and limitation of gas exchange. Desiccation is much more extensive loss of water, which can potentially lead to gross disruption of metabolism and cell structure and eventually to the cessation of enzyme catalyzed reactions (Smirnoff, 1993; Jaleel et al., 2007). It is characterized by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata and decrease in cell enlargement and growth. Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally death of plant (Jaleel et al., 2008).

Attempts were made to transform kufri jyoti, local pahari cultivar of potato vern. Pahari aalu through Agrobacterium tumefaciens mediated transformation for drought
tolerance. GV3101 strain of *Agrobacterium* was utilized for transformation process. Plasmid pBinAR carrying the desired osmotin gene regulated by CaMV 35s promoter and nptII gene as a marker gene was first immobilized in *Agrobacterium* strain by freeze thaw method which was further used for plant transformation. Successful regeneration fascilitated transformation studies. Explants were given small cuts before submerging them in bacterial suspension for 20 min. Wounded tissues produce compounds such as acertosyringone which are known to act as signal molecules responsible for activation of *Agrobacterium* mediated virulent factors. Acertosyringone is known to induce the virulence genes of *Agrobacterium* necessary for effecting transfer and incorporation of T-DNA in host plant (*McCullen and Binns, 2006*). Compounds that induce virulence genes are not normally made by plant, but are produced upon wounding, a prerequisite for plant transformation by *A. tumefaciens*. The use of *Agrobacterium* mediated transformation is a fast and highly efficient method, which does not require expensive equipment. Several methods have been published over the years for various plant species in an attempt to improve transformation efficiency (*Zottini et al., 2008*). 

Osmotin is named so due to its induction by low water potential of the growth medium and a correspondence between the level of osmotin protein produced and the degree of osmotic stress. Osmotin gene was originally isolated from tobacco (*Capelli et al., 1996*). Osmotin is a stress-responsive multifunctional 24-kDa protein and provides osmotolerance to plants. Osmotin, an antifungal cytotoxin, is also a plant pathogenesis-related protein that causes rapid cell death in yeast (*Kupchak et al., 2008*). It also exhibits cryoprotective functions and represents a member of PR5 pathogenesis-related protein family (*Yun et al., 1998; Lee et al., 2010*).
In the present study co-cultivation period of two days was found suitable for infection of explants with *Agrobacterium tumefaciens* with a density of 0.4 O.D at 590 nm for transformation. Kanamycin resistance (50 µg ml\(^{-1}\)) was used as selectable marker for transformants. This property is induced by nptII gene which provides resistance against some antibiotics like neomycin, kanamycin, gentamycin etc. Kanamycin is the most commonly used antibiotic for selection procedure. But the percentage of root induction in putative transformed shoots reduced to 60% in kanamycin containing medium as compared to 100% response in control plants in MS medium. This result concludes some inhibitory effect of kanamycin on regeneration. Cephotaxime concentration of 250 µg ml\(^{-1}\) in the medium was found optimum to control the growth of *Agrobacterium tumefaciens*. Cephotaxime provides a benefit of being lethal to bacteria while at the same time harmless to growing plant (Silva and Fukai, 2001).

Putative transformed plantlets were tested for osmotin gene integration by PCR using gene specific primers. PCR positive samples were further confirmed by sequencing and BLAST of the nucleotide sequence obtained with the osmotin gene sequence in NCBI data base. Samples exhibited 92-97% identity confirming the integration. In bioinformatics, Basic Local Alignment Search Tool (BLAST) is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of different proteins or the nucleotides of DNA sequences. A BLAST search enables a researcher to compare a query sequence with a library or database of sequences, and identify library sequences that resemble the query sequence above a certain threshold.

Results suggested that nodes as explants for transformation followed by direct regeneration has a clear advantage over leaf and internodes via callus phase.
Transformation efficiency obtained through nodes was 6% while that of other explants was only 2%.

Drought stress reduces plant growth by affecting photosynthesis, respiration, membrane stability index (MSI) and nutrient metabolism (Jaleel et al., 2008). Morphological and physiological changes in response to drought stress can be used to help identify resistant genotypes or produce new varieties of crops for better productivity under drought stress (Nam et al., 2001). The reactions of plants to drought stress depend on the intensity and duration of stress as well as the plant species and its stage of growth (Parameshwarappa and Salimath, 2008).

Plants respond to survive under water-deficit conditions via a series of physiological, cellular, and molecular processes culminating in stress tolerance. In drought stress conditions, plants close their stomata to avoid further water loss. Many drought-inducible genes with various functions have been identified by molecular and genomic analyses in Arabidopsis, rice, and other plants, including a number of transcription factors that regulate stress-inducible gene expression. The products of stress-inducible genes function both in the initial stress response and in establishing plant stress tolerance (Shinozaki and Yamaguchi-Shinozaki, 2007).

The transformed plants obtained were subjected to a water stress of five days. On re-watering transgenic, plants recovered from stress and survived, on the other hand, control plants lost growth. This finding indicates better tolerance of transgenic plants towards drought than the wild type.

Drought triggers the production of the phytohormone abscisic acid (ABA), which in turn causes stomatal closure and induces expression of stress-related genes.
Introduction by gene transfer of several stress-inducible genes has demonstrably enhanced abiotic stress tolerance in transgenic plants (Zhang et al., 2004; Bartels and Sunkar, 2005; Umezawa et al., 2006). These particular genes encode key enzymes regulating biosynthesis of compatible solutes such as amino acids (e.g. proline), quaternary and other amines (e.g. glycinebetaine and polyamines), and a variety of sugars and sugar alcohols (e.g. mannitol, trehalose, galactinol, and raffinose).

Plants also respond and adapt to water deficit at both the cellular and molecular levels, for instance by the accumulation of osmolytes and proteins specifically involved in stress tolerance. An assortment of genes with diverse functions are induced or repressed by these stresses (Shinozaki et al., 2003; Bartels and Sunkar, 2005; Yamaguchi-Shinozaki and Shinozaki, 2005). Most of their gene products may function in stress response and tolerance at the cellular level. Significantly, the introduction of many stress-inducible genes via gene transfer resulted in improved plant stress tolerance (Zhang et al., 2004; Umezawa et al., 2006).

Proline accumulation has been reported during conditions of drought (Choudhary et al., 2005). In stressed plants proline accumulation has a protective function, which has been emphasized in numerous reviews (Hare and Cress, 1997; Kishor et al., 2005, Verbruggen and Hermans, 2008). Proline was considered as an inert compatible osmolyte that protects subcellular structures and macromolecules under osmotic stress (Csonka and Hanson, 1991). Osmotic regulation can enable the maintenance of cell turgor for survival or to assist plant growth under severe drought conditions in pearl millet (Shao et al., 2008).

Proline content in leaves of transformed potato plants was significantly high under drought stress (28.02 µM gm⁻¹ fresh weight) as well as under non stress condition.
(13.88 µM gm\(^{-1}\) fresh weight) as compared to wild type under stress (10.87 µM gm\(^{-1}\) fresh weight) and non stress condition (4.46 µM gm\(^{-1}\) fresh weight). This finding indicates towards better adaptability of transformed plants towards drought stress. Proline has been shown to function as a molecular chaperone able to protect protein integrity and enhance the activities of different enzymes (Szabados and Savoure, 2009). Proline metabolism in plants, however, has mainly been studied in response to osmotic stress (Verbruggen and Hermans, 2008). It does not interfere with normal biochemical reactions but allows the plants to survive under stress (Stewart, 1981). The accumulation of proline in plant tissues is also a clear marker for environmental stress, particularly in plants under drought stress (Routley, 1966) and may also be part of the stress signal influencing adaptive responses (Maggio et al., 2002).

Relative water content of transgenic potato plants did not show any significant difference under non-stressed condition as compared to wild type. However, the RWC of transformed potato plants decreased only 4.90% under stress condition while wild type showed a remarkable decrease of about 14.57%. This finding suggests that the transformed plants possess higher water holding capacity under extreme conditions. The effects of drought stress on RWC and leaf water potential have also been investigated in many other studies on grass, *Ctenanthe setosa*, wheat and chickpea. (Jinmin and Huang, 2001; Terzi and Kadioglu, 2006; Bayoumi et al., 2008; Rahbarian et al., 2011). It is believed that leaf water potential and RWC are reliable parameters for quantifying the plant drought stress response (Siddique et al., 2000; Bayoumi et al., 2008).

Most studies have shown decreased relative water content and leaf water potential in response to drought stress. Genotypes that maintain higher relative water content under drought stress are believed to be more tolerant and give higher yield than others (Bayomi
et al., 2008). It has also been observed generally that genotypes with higher leaf water potential and relative water content have a higher photosynthetic rate under drought stress (Siddique et al., 2000). These parameters can serve as useful markers for screening chickpea genotypes and identifying drought-tolerant genotypes (Jaleel et al., 2009).

The overall analysis of various parameters observed under different conditions confirms the transformation with osmotin gene as an effective approach imparting drought tolerance in potato plants.
SUMMARY AND CONCLUSION