CHAPTER 7

REVIEW OF LITERATURE

Cell culture techniques have many potential advantages over those traditionally used for plant breeding. These are

1. large variations within genotypes may be generated during culture.
2. large number of genotypes can be evaluated and selected in the laboratory using relatively little space.
3. time between generations can be reduced.
4. the environment and nutrient conditions can be uniformly and precisely controlled.
5. cells growing in culture are uniform in development, thus complications due to differences in morphology and stage of development are reduced.
6. Traits can be selected at the cell level (i.e., salt tolerance) or somaclonal variability for that trait can be evaluated in regenerated plants and their progeny.

However, there might be disadvantages to use cell culture techniques, particularly when the integrated functioning of the whole plant is an essential part of its ability to tolerate high salt concentration (Dracup, 1991). In programmes focused on the incorporation of potential genotypes into a breeding scheme, plants must be regenerated from cultured cells. In several species, repeated subculture of callus and suspension cultures leads to a decline or loss of regenerative capacity, it may be that tolerant cell cultures can be selected but that they are, by then, non-morphogenic.

Cell lines exhibiting tolerance to salt have been isolated from more than 33 plant species of 25 genera belonging to 13 families (Table 1). Most of the species are from three families, i.e. Solanaceae -7, Poaceae -6 and Fabaceae -5. The following conclusions can be drawn from Table 1.

1. Selective agent : In most of the studies, NaCl was used as the selection agent. Several investigators have also employed other salts, e.g. Na_2SO_4 (Pua and Thorpe, 1986), artificial sea water (Nyman *et al.*, 1983), sea water (Yano *et al.*, 1982) or soil solution (Mc Hughen and Swartz, 1984) and salt mixture (Chen *et al.*, 1980). A number of studies have used osmotic selective agents such as polyethylene - glycol (PEG), dextran, mannitol or melibiose (Goldner *et al.*, 1977; Chen *et al.*, 1980; Handa *et al.*, 1982a, 1983; Binzel *et al.*, 1985; Dracup and Greenway, 1988). These studies have pointed out the contribution of water stress to the effects of salts during selection and in studies of osmotic adjustment in the absence of ion toxicity.

2. In most of the experiments, callus tissue or cells in suspension / cell clumps plated onto solid media have been used for selection. However, protoplast derived callus has not been used so for. In some cases, the selection system included various explants from which callus (Nabors *et al.*, 1982; Yano *et al.*, 1982; Lebrun *et al.*, 1985; Li *et al.*, 1987); embryos (Lebrun *et al.*, 1985) or plantlets (Mathur *et al.*, 1980, Li *et al.*, 1987, Jain *et al.*, 1991a) were induced to form directly in saline media. But, the use of the tissue culture techniques such as somatic hybridisation, embryo rescue and pollen / anther culture has not been given due consideration (Chandler and Thorpe, 1986).

3. Most of the cells exposed to salt was diploid or polyploid and only very few of them were haploid (Zenk, 1974; Dix and street, 1975; Croughan *et al.*, 1981; Tyagi *et al.*, 1981). In diploid and polyploid cells only dominant or co-dominant nuclear mutation or mutations in organelles can be expressed phenotypically, whereas in haploid cells recessive mutation can also be expressed. However, comparative studies have shown that no differences were found between haploid and diploid cells in the rate of occurence of resistant lines (Croughan *et al.*, 1981).

4. In majority of the cases spontaneous variability appears to be sufficient to allow for effective selection. Mutagen treatments prior to selection have been employed to increase mutation frequency in some cases (Nabors *et al.*, 1975; Kochha *et al.*, 1982; Gosal and Bajaj, 1984), but no increase in the recovery rate of resistant lines above the spontaneous rate was detected except in the one case (van Swaaij *et al.*, 1986). McCoy (1987 b) showed that NaCl may enhance somaclonal variations.

5. In almost all the cases selection medium contained growth regulators, however, in one case (McHughen and Swartz, 1984), the selection media contained no growth regulators. McHughen and Swartz (1984) pointed out that growth regulators can influence the response of the cells to salt and, hence, may lead to misinterpretation of the results. A possible effect of the medium constituents on the response of the cells to salt stress was suggested by Flowers *et al.*, (1985) as an explanation for the unexpected higher salt resistance of cultured rice cells compared with that of leaf cells.

6. In vitro selection procedure : The isolation of salt-resistant cell lines has been done by exposing the small pieces of callus or by plating the cell suspension to a lethal concentration of salt(s) (direct selection) or by gradual stepwise increase in salt(s) at each subcultures (stepwise selection). The excess salt(s) has been included as an additional component of the agar modified nutrient medium. The resistant cells are identified by their continued growth on otherwise inhibitory level of salt(s). McHughen and Swartz (1984) used the direct selection system, as it more closely resembles the situation in the field where seeds are planted directly into, and therefore, immediately encounter, the saline environment. The gradual imposition of stress

will be inefficient in that it will more readily select for physiological adaptations as non-tolerant cells with a labile metabolism will have time to adapt to the gradually imposed stress, and will, therefore, be positively selected. A similar opinion was expressed by Chandler and Vasil (1984). On the other hand, Harms and Oertli (1985) preferred the stepwise selection, since the one-step procedure primarily tests the ability of cells to tolerate and to recover from osmotic shock rather than their ability to withstand a certain level of salt or osmotic shock. According to them, the direct shift to high stress conditions does not leave sufficient time for the cells to express their inherent capacities for stress response and adaptation which can be remarkable. Bowman (1987) suggests that genetic differentiation with respect to growth and survival is expressed better under a rapidly induced short-term salinity treatment rather than gradually imposed salinity.

7. Indirect selection: (a) Selection for proline overproducing (by selection for resistant to proline analogues) has been investigated as a possible alternative strategy for the production of salt-resistant plants. Increased salt-resistance in such mutant cell lines has been demonstrated for several plant species, i.e. *Hordeum vulgare* (Kuch and Bright, 1981), *Ducus carota* (Riccardi *et al.*, 1983), *Nicotiana sylvestris* (Dix *et al.*, 1984), *Solanum tuberosum* (van Swaaji *et al.*, 1986), *Brassica napus* (Chandler and Thorpe, 1987), *Arabidopsis thaliana* (Lehle *et al.*, 1989) and *Vigna radiata* (Kumar and Sharma, 1989b).

b. Another approach to salt-resistant cells has been based on selection of cells resistant to only the osmotic stress component of salinity. Osmotically adapted cells offers a unique possibility to separate specific ion effects from osmotic effects experimentally. Moreover, these cells can be exposed to conditions of high ionic stress without being osmotically shocked (Harms and Oertli, 1985). They suggested two stage selection procedure, the cells are first exposed to non-penetrating osmoticum for the selection of osmotically adapted cells and then to salt for selection of cells resistant to ionic stress. The compounds used as first-step osmoticum in such experiments should be non-penetrating and non-toxic. The finding of Thompson *et al.*, (1986) that mannitol is taken up and metabolised in *Daucus carota* and *Nicotiana* cells makes its applicability doubtful. Polyethylene - glycol (Handa *et al.*, 1982a, 1983) and melibiose (Dracup *et al.*, 1986) were found suitable for this purpose. Osmotically adapted cell cultures of *Lycopersicon esculentum* (Bressan *et al.*, 1981; Handa *et al.*, 1982a, 1983), *Daucus carota* (Harms and Oertli, 1985) and *Solansum tuberosum* (Sabbah and Tal, 1990) showed an increased resistance to salt stress as compared with non-adapted cultures.

8. Quantification of tolerance: Tolerance can be best defined as the property by virtue of which the selected lines remain viable and grows at salt concentration completely inhibitory to unselected cells. Salt tolerance has generally been determined by measuring the increment of fresh or dry weight in callus and cell-suspensions or by measuring packed cell volume or cell

number in cell-suspension. Visual appearance is an additional aspect for the discription of callus growth. Some precautionary measures were suggested while measuring growth in tissue or cell culture. These include : correction for the volume of the free space when growth is determined in terms of volume. Like wise determination of growth based on fresh or dry weight requires correction for the weight of solution or solutes in the free space of cell pellets. Dracup *et al.,* (1986) washed cell pellets with ^{14}C sorbitol solution isotonic with the culture medium and found that solution in the free space accounted for 50-60% of the fresh weight of pellets (hence a similar percentage of pellet volume). Further more, solutes in the free space would contribute over 20% of the dry weight and this would rise with increasing concentrations of solutes in the medium, such as high NaCl or other osmotica. Failure to adjust for free space would be more serious when comparing absolute growth than when comparing relative growth rates, particularly if treatments affect cell size (Dracup, 1991).

9. Retention of salt tolerance : Many researchers have evaluated salt selected cell lines to see if the tolerance to salt was stable after the cells had been grown away from salt for several passages. Almost all of them found that the slected tolerant cells maintained their tolerance to salt during mitotic divisions in the salt free media. Stability of tolerance after growth in medium without salt has been suggested as an indicator for selection of a true genetic variant (Dix and Street, 1975; Mathur *et al.,* 1980; Tyagi *et al.*, 1981; Ranjan and Vasil, 1983; Pandey and Ganapathy, 1984; Watad *et al.,* 1985; Bresson *et al.,* 1985, 1987). In one case, *Nicotiana tabacum* selected cells lost their tolerance after being grown for five cell mass doublings in medium without NaCl (Hasegawa *et al.*, 1980).

Lack of stability indicates that cell became tolerant as a result of physiological adaptation (Watad *et al.*, 1985). Bressan *et al.*, (1985) suggested that the stability observed in their material is the result of enrichment of the cell population with pre-existing cells having a stable resistance to high salt levels. The exact meaning of stability will probably be clarified only when the mechanisms responsible for its expression are known. At the present stage, the critical and most reliable test for the genetic basis of salt resistance in cultured cells remains the regeneration of salt-resistant somaclones and the demonstration of sexual inheritance of this characteristic (Tal, 1991).

10. The concentration of salt used for selection varied considerably. The highest reported NaCl concentration to which cells were able to adapt was 770 mol m^{-3} (Hasegawa *et al.*, 1986), which is much higher than the NaCl concentration in seawater. Bressan *et al.*, (1985) suggested that in the tobacco cells they studied, resistance to a moderate level of NaCl (171 mol m^{-3}) results from a totally reversible adaptation, while resistance to higher levels (428 mol m^{-3}) of NaCl results from both a reversible adaptation and a selective enrichment of the cell population with

pre-existing, stable, non-reversible resitant cells. The question of whether a threshold concentration above which enrichment of cells of the latter type occurs, also exists in other species should be clarified. The possibility that manipulation of other factors besides salt concentration may help to distinguish between true mutants and adapted cells, should not ruled out. For example, Henke (1981) suggests selecting mutants in a single step, and epigenetic variants stepwise.

11. Cell cultures tolerant to NaCl may show cross-tolerance to polyethylene glycol (Chandler and Vasil, 1984; Ben-Hayyim *et al.*, 1987) or mannitol (Stavarek and Rains, 1984) or various sulfate salts ($MgSO_4$, Na_2SO_4, K_2SO_4, Kochba *et al.*, 1982) or cross-sensitivity to KCl (Ben-Hayyim and Kochba, 1983) or no cross-tolerance (Pandey and Ganepathy, 1984). Na_2SO_4 tolerant callus of sugarbeet showed cross-tolerance to NaCl (Pua and Thorpe, 1986).

12. Plant regeneration via organogenesis and somatic-embryogenesis from salt-tolerant cells has been exceedingly difficult in most of the cases, by the time, the cell selection was made, the cells had lost their regeneration capacity (Komizarko and Khretonova, 1973; Smith and McComb, 1983; Bhaskaran *et al.*, 1983; Ranjan and Vasil, 1983; Chandler and Vasil, 1984; Bajaj and Gupta, 1987). In some species, chromosomal abberations were found to accompany the loss of regenerability (McCoy, 1987 b). According to Croughan *et al.*, (1981), the loss of regeneration capability seems to be a technical problem, which can be overcome by development of appropriate media sequence. The approaches commonly used to overcome the problems encountered in regeneration include the manipulation of hormone (Stavarek *et al.*, 1980, Tatchell and Binns, 1986), carbohydrates (Lupotto, 1983; KaviKishor, 1987), which can be used as energy source or as osmotica, and Na_2SO_4 (Pua *et al.*, 1985). The latter two may exert their effect indirectly by influencing the endogenous hormonal balance. Moreover, even the presence of salt, with few exceptions (Tyagi *et al.*, 1981), is inhibitory to regeneration, especially by somatic embryogenesis (Ranjan and Vasil, 1983; Chandler and Vasil, 1984). Regeneration, therefore, must be accompanied in the absence of selection pressure (Nabors *et al.*, 1980; Chandler and Vasil, 1984; Ranjan and Vasil, 1983; Tyagi *et al.*, 1981; Yano *et al.*, 1982). This enables potential regeneration from not only tolerant cells, but also unadapted / non-tolerant cells freed from the constraint of salt in the medium. Sometimes, NaCl even enhances embryogenesis (Labrun *et al.*, 1985; Spiegal-Roy and Ben-Hayyim, 1985). Additional possible approaches to overcome the regeneration problem are : (1) decreasing the time of exposure of the cells to salt by a shortterm selection (McHughen and Swartz, 1984); (2) producing calli or suspension cells directly in saline media (Nabors *et al.*, 1982; Yano *et al.*, 1982; Lebrun *et al.*, 1985); (3) differentiating embryo or plantlets from various explants directly in

saline media (Mathur *et al.*, 1980; Lebrun *et al.*, 1985; Li *et al.*, 1987; Jain *et al.*, 1991a); (4) using cells which have a high inherent capacity for regeneration (Ranjan and Vasil, 1983; Smith and McComb, 1983; Chandler and Vasil, 1984).

13. Testing of salt tolerance in regenerants and its sexual inheritance : Whole plant evaluation of regenerated material is the critical step in determining the efficiency of *in-vitro* selection. Plant regeneration from salt-tolerant cell lines were not salt tolerant in *Medicago sativa* (Smith and McComb, 1983) and *Pennisetum purpureum* (Chandler and Vasil, 1984). Bhaskaran *et al.*, (1986) noted in a hydroponics system testing their sorghum derived from a salt tolerant line, an increase in saline stress resulted in a decrease in productivity in both control and experimental lines, but that the selected line appeared more vigorous than the parent under non-stressed conditions. Spiegel-Roy and Ben-Hayyim (1985) tested this putative salt tolerant *Citrus* plantlets in culture medium. McHughen (1987) studied the performance of progeny of flax plants regenerated from a salt tolerant cell line in saline stressed and normal soil and observed putative salt tolerant line was superior in saline soil than parental line (McHughen, 1987), but these salt tolerant lines of flax were found fail in recent intensive field tests (Rowland *et al.*, 1989). Similarly, regenerated salt tolerant cell lines of *Medicago sativa* grew poorly in comparison to parental type and the only plant that flowered was both male and female sterile (McCoy, 1987 b).

TCCP group tested the performance of whole plants of sorghum, rice and wheat regenerated from salt tolerant cells. This group reported that longer the material is maintained *in-vitro* and the higher the level of *in-vitro* stressing agent, the higher the probability of genetic events which result in reduced performance or sterility. Results also indicated that there is higher probability of improving the stress tolerance of previously non-tolerant cultivars than of improving tolerance of cultivars with higher levels of inherent tolerance.

Inheritance of salt tolerance appears stable through several seed generation in *Nicotiana tabacum* (Nabors *et al.*, 1980), *Avena sativa* (Nabors *et al.*, 1982), *Oryza sativa* (Vajrabhaya *et al.*, 1989), *Beta vulgaris* (Freytag *et al.*, 1990), *Brassica juncea* (Jain *et al.*, 1991b; Kirti *et al.*, 1991), *Medicago sativa* (Winicov, 1991) and *Nicotiana plumbaginifolium* (Sumaryati *et al.*, 1992). In *Nicotiana*, the genetic changes was interpreted as being due to a dominant or co-dominant mutation although the mechanism of inheritance appeared not to be Mendelian (Nabors *et al.*, 1980). The salt tolerance in regenerated plants of *Medicago sativa* (Winicov, 1991) and *Nicotiana plumbaginifolium* (Sumaryati *et al.*, 1992) appeared to be transmitted as a single dominant gene mutation. The genetic analysis of additional cases, in various plant species, is required before a final conclusion can be made on the genetic control of salt tolerance in regenerated plants.

Callus vs whole plant

Several researchers have compared the performance of plants and their corresponding callus under salt stress. Von Hendenstron and Breckle (1974) studied the growth of *Suaeda maritima* and *Salicornia europaea*. Both the plants and callus showed improved growth with the addition of NaCl. Tal *et al.*, (1978) looked at the response of *Lycopersicon esculentum*, *L. peruvianum* and *Solanum pennellii* to 0-2% NaCl. They found that the callus responsed in a similar fashion as the whole plant, the mild species (*L. peruvianum* and *Solanum pennelli*) had better growth under salinity than cultivated species (*L. esculentum*). Orton (1980) compared the salt tolerance of *Hordeum vulgare* and *H. jubatum* in whole plants and callus cultures. The callus responses paralleled the plant as NaCl was increased to 0.17 M. On the other hand, Smith and McComb (1981) making similar comparison among a salt-sensitive glycoplyte (*Phaseolus vulgaris*), a salt-tolerant glycoplyte (*Beta vulgaris*), and two haloplytes (*Atriplex undulata* and *Suaeda austrilis*) found that both the plant and callus of the salt sensitive glycophyte decreased in growth with increasing salt. The salt-tolerant glycophyte, as to both plant and callus, showed increased growth at intermediate salt levels, but a decrease with higher levels. The haloplytic plants showed increased growth with higher levels of salt, while that of the callus decreased. They suggested that salt tolerant glycophyte has cellular level mechanism for salt tolerance, while that of halophyte depends on the strucutre of the whole plant. Another halophyte, *Distichlis spicata* also is more resistant in culture than two glycophytic species *Nicotiana sylvestis* and *Zea mays* (Warren and Gould, 1982). Dains and Gould (1985) also suggested cellular basis of resistance in halophytes.

Smith and McComb (1981 b) have also examined the response of increasing NaCl of three pasture legumes and the corresponding callus cultures. They found very similar responses between the plant and callus and suggested that the use of callus cultures to screen for NaCl tolerance is a valid system. On the other hand, McCoy (1987a) observed no significant positive relationship between growth in the whole plant and callus on salt media. Similar views are also expressed by Smith *et al.*, (1989) that the selection based on the apparent NaCl tolerance of callus tissue or male gametophyte would appear unproductive in *Medicago sativa*. Thus, the positive correlation, where both the whole plant and the cultured cells are resistant to salt, is interpreted as an indication for the operation of cellular mechanisms of salt resistantce on the whole plant level. A negative correlation, where the whole plant is salt-resistant, but the isolated cells are sensitive, was regarded as an indication for the operation of mechanisms depending on the organization of the cells in tissue and/or organs in the whole plant. One of the unsolved dilemmas in this respect is the different response of cultured cells of different halophytes to salt in culture media; some are resistant and some are sensitive. There seems, however, a correlation between this response and the type of medium used : sensitive cells were found on

solid medium, resistant in liquid medium. Whether or not this correlation is causal is still an open question (Tal, 1990). Another negative correlation, where the plant is sensitive but the isolated cells are resistant, was found in rice (Flowers *et al.*, 1985) and beans (Gale and Boll, 1978).

Table 1 : Variation for salt resistance from cultured tissues and cells of various plant species

	Species	Type of culture	Salt(s)	Concentration of salt (mol m^{-3})[a]	Mutagen used	Exposure to salt OS=one step G=Gradual	Stability in salt free medium T=no.of transfers G=no.of generations	Regeneration (R_0)	Resistance of R_0	Sexual transmittance	Reference
Apiaceae											
1.	Daucus carota	Callus						+ (Plants)	+		Komizerko and Khretonava, (1973)
2.	D. carota	Callus	Sea water or various salts								Goldner et al. (1977)
3.	D.carota	Suspension	DR-->SR			G					Harms and Oertli (1985)
Araceae											
4.	Colocasia esculenta	Callus and suspension	Synthetic sea water			G		+ (plantlets)			Nyman et al. (1983)
Brassicaceae											
5.	Brassica oleraceae							Plantlets			Wardle et al. (1981)
6.	B.napus	Callus	Na_2SO_4	176		OS and G		+ (roots)			Chandler et al. (1986)
7.	B. napus	Callus	Na_2SO_4	140			+ (13 T)				Chandler and Thorpe (1987)
8.	B.juncea	Cotyledon	NaCl	128-171				+ (Plantlets)	+	R_0 and R_1	Jain et al. (1991)
9.	B.juncea	Somatic embryos	NaCl	214				+ (Plantlets)	+	R_0 and R_1	Kirti et al. (1991)
Chenopodiaceae											
10.	Beta vulgaris	Callus	NaCl Na_2SO_4	210 and 560		G					Pua and Thorpe (1986)
11.	Beta vulgaris	Petioles from in-vitro multipled shoots	NaCl Na_2SO_4 $NaHCO_3$ $CaCl_2$ $CaSO_4$	20.5 14.0 19.0 10.8 11.7				+ (Plantlets)	+	+	Freytag et al. (1990)
Convolvulaceae											
12.	Ipomea batata	Suspension	NaCl	171		OS	+ (3 T)				Salgadogarciglia et al. (1985)
Fabaceae											
13.	Cicer arietinum Pisum sativum Vigna radiata	Callus and suspension	NaCl	427	EMS	OS and G		+ (roots)			Gosal and Bajaj (1984)

14.	C. arietinum	Callus	NaCl	100		OS	+ (3T)				Pandey and Ganapathy (1984)
15.	Glycine max	Callus (haploid)	$NaNO_3$	64							Jai-Ping et al. (1981)
16.	Medicago sativa	Suspension	NaCl	171		OS		+ (Plants)			Croughan et al. (1978); Stavarek and Rains (1984)
17.	M. sativa	Suspension	NaCl					+ (Plants)	-		Smith and McComb (1983)
18.	M. sativa	Callus	NaCl	85 and 171		G	+ (4T)	+ (plants)	+		McCoy (1987b)
19.	M.sativa	Callus	NaCl	171		OS	+	+ (plants)	+	+ (R_0,R_1,R_2)	Winicov (1991)
20.	Vigna aconitifolia	Callus	NaCl	171			+				Bhargava and Chandra (1989)
21.	V.radiata	Callus	NaCl	300		OS	+ (3T)				Kumar and Sharma (1989)
Linaceae											
22.	Linum usitatissimum	Callus	Artificial soil solution			OS		+ (Plants)	+	+	McHughen and Swartz (1984)
Poaceae											
23.	Avena sativa	Callus	NaCl	171		G		+ (Plants)		F_1 and F_3 fertile	Nabors et al. (1982)
24.	Oryza sativa	Callus and suspension (haploid and liploid)	NaCl	256 and 342	-	OS					Croughan et al. (1981); Starark and Rains (1989)
25.	O. sativa	Callus	Sea water			OS		+ (Plants & seeds)	+	F_1 sterile	Yano et al. (1982)
26.	O.sativa	Callus	NaCl	307				+ (plantlets)			Yasuda et al. (1980)
27.	O.sativa	Callus	NaCl	342	M	OS	-	+ (Plants & few seeds)	+	+	Wong et al. (1983)
28.	O.sativa	Callus	NaCl	342	M	OS		+ (Plants)		+	Woo et al. (1985)
29.	O.sativa	Embryogenic callus	NaCl	171 or 342		OS		+ (Plants)	+	+ (R_1, R_2)	Vajrabhaya et al. 1989
30.	Pennisetum americanum	Embryogenic suspension	NaCl	198		G	+ (7T)	+ (Embryoids)			Rajan and Vasil (1983)
31.	P. americanum	callus	NaCl	171		OS	+	roots and embryoids	+		Bajaj and Gupta (1987)
32.	Pennisetum purpureum	Embryogenic callus	NaCl	214 and 342		OS and G	-	+ (Plants)			Chandler and Vasil (1984)

33.	P.purpureum	Embryogenic callus						+ (Plants)	+	Ashton (1986); Hilderth (1986)
34.	P.purpureum	Callus	NaCl	342		OS	+	+ (Plants)	+	Bajaj and Gupta (1986, 1987)
35.	Saccharum officenarum	Suspension and Callus	NaCl	257		G				Liu and Yeh (1982)
36.	S.officenrarum	Suspension and callus	NaCl	257						Yasuda et al. (1980)
37.	S.officinalis	Callus	NaCl	257				+ (Plants)		Naik and Babu (1988)
38.	Sorghum bicolor	Callus	NaCl	86		OS		+ (Plants & few seeds)	+?	Bhaskaran et al. (1983,1986)
39.	Triticum aestivum	Embryogenic callus	NaCl	103		G		+ Plants		Faizi and Ferguson (1986)
Rosaceae										
40.	Colt Cherry	Callus and protoplasts	NaCl, KCl and Na_2SO_4	200		OS		+ plants	+	Ochatt and Power (1988)
Rutaceae										
41.	Citrus aurantium	Callus and suspension	NaCl	150	γ radation	G	+ (3T)	+ (embryoids)		Kochba et al. (1982); Spiegel-Roy and Ben-Hayyim (1985)
42.	C.sinensis	Callus and suspension	NaCl	200	γ radiation	G	+ (3T)	+ (embryoids and plantlets)		Kochba et al. (1982); Spiegel-Roy and Ben-Hayyim (1985)
Scrophulariaceae										
43.	Kickxia ramosissima	various explants	NaCl	240			+ (3T)	+ (Plants with flowers)		Mathur et al. (1980)
Solanaceae										
44.	Capsicum annum	Callus and suspension	NaCl	171 and 342		OS	+ (3T)			Dix and Street (1975)
45.	Datura innoxia	Callus (Haploid)	NaCl	171		OS	+ (1 month)	+ (Plants)	+ (Callus)	Tyagi et al. (1981)
46.	Nicotiana Sylvestris	Suspension (Haploid)	NaCl	171		G				Zenk (1974)
47.	N. sylvestris	Callus and suspension (Haploid and Diploid)	NaCl	171 and 342		OS	+ (3T)			Dix and Street (1975)

48.	*N. tabacum*	Suspension	NaCl	150	EMS	G		+ plants	+	+	Nabors et al. (1975, 1980)
49.	*N. tabacum*	Suspension	NaCl	171		G	-(IT)				Hasegawa et al. (1980)
50.	*N. tabacum*	Suspension and callus	NaCl	130 (SR-->DR)		G					Heyser and Nabors (1981 a,b)
51.	*N. tabacum*	Suspension	NaCl	200 and 500		G	+ (24 G and 56 G)				Watad et al. (1983, 1985)
52.	*N. tabacum*	Suspension	NaCl	600 and 770		G					Binzel et al. (1985); Hasegawa et al. (1986)
53.	*N. tabacum*	Suspension	NaCl	428		G	+ (100 G)	+ plants	+	+	Bressan et al. (1985, 1987)
54.	*N. tabacum*	Callus	Na_2SO_4	70		OS		+ (Plant)	+	+	Pua et al. (1985); Pua and Thorpe (1986)
55.	*N.plumbaginifolium*	Protoplasts (haploid)	NaCl	188				+ (Plant)		+ (F_2)	Sumaryati et al. (1992)
56.	*Lycopersicon esculentum*	Suspension	DR-->SR								Bressan et al. (1981)
57.	*L. esculentum*	Cotyledon	NaCl	128		OS		+ (shoots)			Li et al. (1987)
58.	*L. esculentum*	Callus	NaCl	342				+ (Plantlets)			Garcia-Reina et al. (1988)
59.	*L. esculentum*	Callus	NaCl	80		G	+ (2G)	+ (roots and shoots)			Rahman and Kaul (1989)
60.	*L. peruvianum*	Suspension	NaCl	350			+ (3T)				Hassan and Wilkins (1988)
Vitaceae											
61.	*Vitis rupestris*	Callus, suspension and embryos	NaCl	150		OS and G	+ (3T)	+ (embryos)			Lebrun et al. (1985)

[a] The concentration given is either the only one used or the upper limit found in series of concentrations; M - mutagenic treatment; SR --> DR and DR --> SR - salt adapted cells were exposed to drought stress and vice-versa, respectively.