The Influence of Calcium and Low Temperatures on Oryzalin-induced Reactions of Wheat Roots – Physiological and Biochemical Aspects.[+)]

Elena Asafova*[)], Olga Timofeeva, Olga Olinevich and Ludmilla Khokhlova

State University of Kasan, Faculty of Biology, Department of Plant Physiology and Biotechnology

*[)] Corresponding author; e-mail: Elena.Asafova@ksu.ru

[+)] Translated by L. Bender

Abstract

It was shown that the inhibitor of tubulin protein polymerization, oryzalin (10 µM) evoked structural changes in the principal part of microtubules in cells of wheat seedling roots and brought about an increase in electrolyte exosmosis from the root tissue as well as an increase in cell wall lectin activity while soluble lectin activity and soluble protein content were decreased. After cold hardening of the seedlings at +3 °C for 7 days an increase of the spatial microtubule aggregation and a decrease of the oryzalin effect were noted. Exogenous $CaCl_2$ (1mM) and the antagonist of the Ca^{2+}-calmodulin complex, chlorpromazin (250 µM) influenced, possibly because of changes in the phosphorylation of proteins interacting with the microtubules and the membraneotrope action of these substances.
It is assumed that the change of cytoskeleton stability brought about by the influence of the external stimuli is linked to the functioning of the cell signaling system, thus defining the biochemical and the physiological reactions of the plants.

Introduction

Calcium is one of the most important regulator ions in cell metabolism. Supposedly, Ca^{2+} ions may act as a link between „soluble“ signals and the cytoskeleton configuration (Fulton,1987). It is known that the maintainance of the cytoskeleton structure and its function depend on the Ca^{2+} concentration in the cytosol (Schliwa et al., 1981; Williamson, 1987).

Microtubules are reported to be stable if the Ca^{2+} concentration in the cells is below 1 μM while mM concentrations initiate rapid destruction of the tubulin proteins (Schliwa et al., 1981). The changes of the structural and polymerization state of the cytoskeleton filaments are brought about by association proteins which act through the activation of the Ca^{2+} -calmodulin complex, i.e. phosphorylation processes (Cyr, 1991).
Recent models suggest that cells contain a uniform system of functional structures which links plasmalemma and cortical microtubules (Sonobe and Takahashi, 1994) as well as lectins and the cytoskeleton (Timofeeva et al., 1999). It is assumed that the stability of cortical microtubules increases if they interact with transmembrane proteins (Loyd et al., 1996). These components ensure signal transduction within the cell where these signals will be transmitted to cellular lectins and proteins. It is now acknowledged that the cytoskeleton is an important factor within the signal transmitting systems of plant cells (Nick, 1998), where the linkage of microtubules to the plasmalemma possibly acts as the target for the cellular signaling system. Traditionally, specific microtubule and microfilament inhibitors are used in studies on the role of the cytoskeleton in cellular processes. In the investigations reported here we used the highly specific inhibitor of the polymerization of plant tubulins – oryzalin which belongs to the chemical group of dinitroaniline herbicides (Morejohn et al., 1987). Still, it is not known whether this inhibitor acts through direct influences on the cytoskeleton structure or via the regulation of cytosolic calcium. The aim of our investigations was to study the permeability of the plasmalemma, the activity of lectins and the content of soluble protein in connection with structural modifications of the cytoskeleton and the Ca^{2+}-calmodulin system under the influence of oryzalin on plants at optimal and suboptimal temperatures.

Material and methods

The studies were carried out using roots of wheat seedlings (Triticum aestivum L., cultivar Mironovskaya 808). The seedlings were incubated for 7 days in either bidistilled water or 1 M $CaCl_2$ at 23 ± 2 °C and a 12 hour photoperiod (100 watts m^{-2}). Cold treatments were also performed for 7 days at 3 ± 1 °C. The antagonist of the Ca^{2+}-calmodulin dependend reaction, Chlorpromazin (0.25 mM) was applied to the medium 24 hours before the cold treatment commenced. Excised roots were incubated in 0.01 mM oryzalin for 3 hours.

The visualization of microtubules in cells of the differentiation zone (6 – 7 mm behind the root tip) was carried out employing indirect immunofluorescence microscopy according to Baluska et al. (1997) using Stidman wax (low melting point) and primary monoclonal α-tubulin antibodies (Amersham, N 356; Sweden) as well as secondary antibodies – anti-mouse immunoglobulin conjugated with biotin (Amersham, RON 1001). Streptawidin-fluorescin (Amersham, RPN 1232) was used as a fluorescent dye. 25 preparations per treatment were investigated employing the fluorescence microscopy system Leica DMLB (Germany) using the software Image-Pro-Plus (DELL Optiplex GX1p). Stability of microtubules was expressed as the percentage of preparations showing polymerized microtubules after the incubation with oryzalin.
The permeability of the plasmalemma was determined conductometrically after incubating wheat roots in distilled water (25 mg root material per 25 ml of water for 2 hours at constant agitation with a magnetic stirrer). Electrical conductivity of the incubation solution was expressed as a percentage of maximum electrolyte exosmosis obtained with root tissue incubated at 100 °C for 30 minutes.
Soluble lectins were extracted with 0.05 M HCl (tissue : medium ratio 1 : 10) for 1 hour. After centrifugation, 1 M Na-phosphate buffer pH 7.2 – 7.4 was added to the supernatant (extract : buffer ratio 2 : 1). This solution was used for the determination of soluble lectin activity. The cell wall bound lectins were recovered from the remaining pellet reextracting it for 4 hours with a medium consisting of one volume of 0.2 M sucrose + 0.02 M KH_2PO_4 + 0.01 M EDTA and six volumes of 0.05 % Triton-X + 0.9 % NaCl (pH 5.0). Here, tissue : medium ratio was 1 : 2. Lectin activity in the supernatants was assayed by the agglutination of trypsin treated erythrocyte preparations and determined as the minimum protein concentration yielded through agglutination. The concentration of soluble proteins and total protein content were measured following the methods of Kumar et al. (1983) and Bradford (1976), respectively. All experiments were carried out in triplicate and the results treated statistically.

Results

In roots of wheat seedlings oryzalin increased the permeability of the plasmalemma by 11 % (Figure 1A) and the activity of the cell wall lectins by 45 % (Figure 2A) at optimal temperatures. These results reflect the changes of microtuble structure in the root cells. Figure 3A shows the structural changes of native microtubules under the influence of this inhibitor which reduces the preparations exhibiting polymerized tubulin structures to 5 % of that found in the control treatments (Table 1). It could also be demonstrated that the activity of the soluble lectins decreased by about 70 % (Figure 4A) and the content of soluble protein by 88 % (Figure 5A).

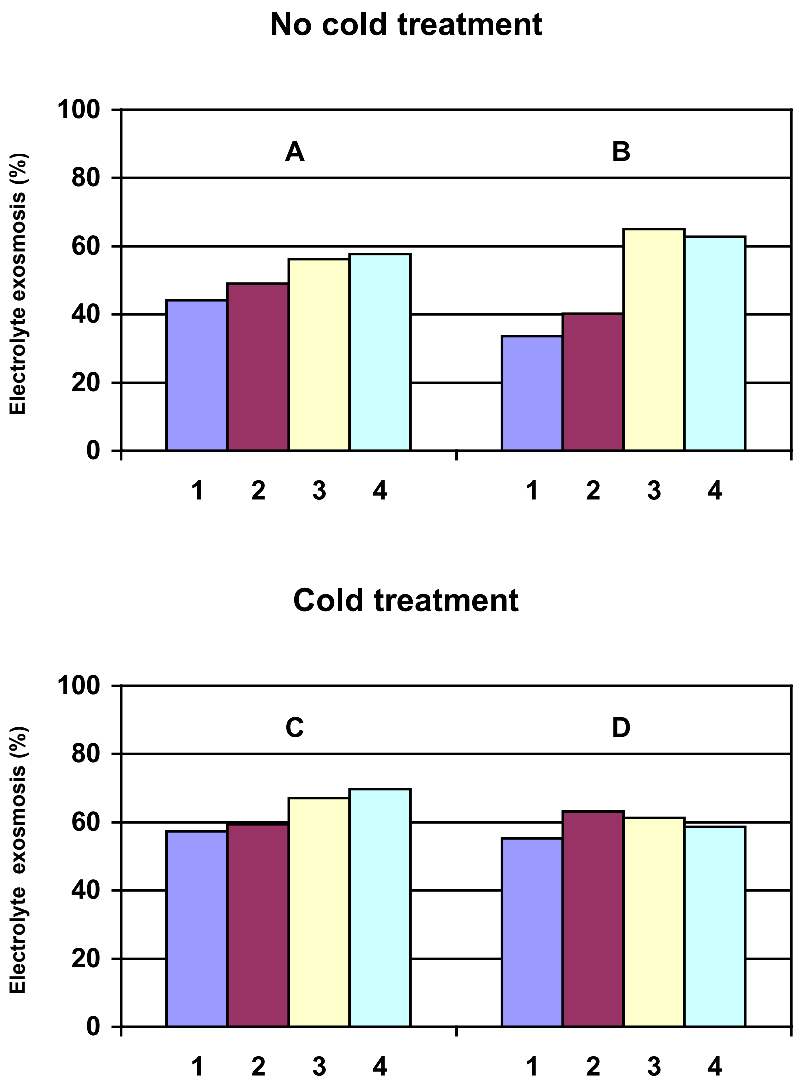

Figure 1: Plasmalemma permeability in roots of wheat seedlings as influenced by oryzalin and chlorpromazin: A, C, without Ca^{2+}; B, D, with Ca2+; 1, control; 2, oryzalin (10 µM); 3, chlorpromazin (250 µM); 4, oryzalin + chlorpromazin.

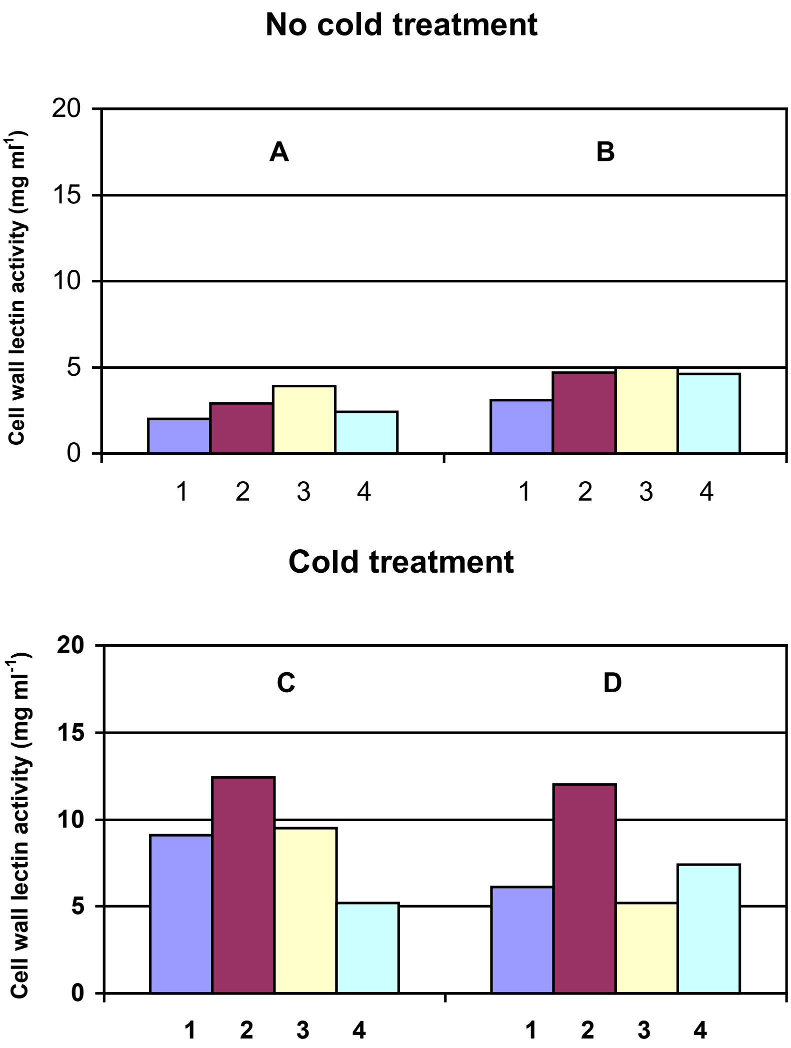

Figure 2: Cell wall lectin activity in roots of wheat seedlings as influenced by oryzalin and chlorpromazin: A, C, - Ca^{2+}; B, D, + Ca^{2+}; 1, control; 2, + oryzalin (10 µM); 3, + chlorpromazin (250 µM); 4, + oryzalin + chlorpromazin.

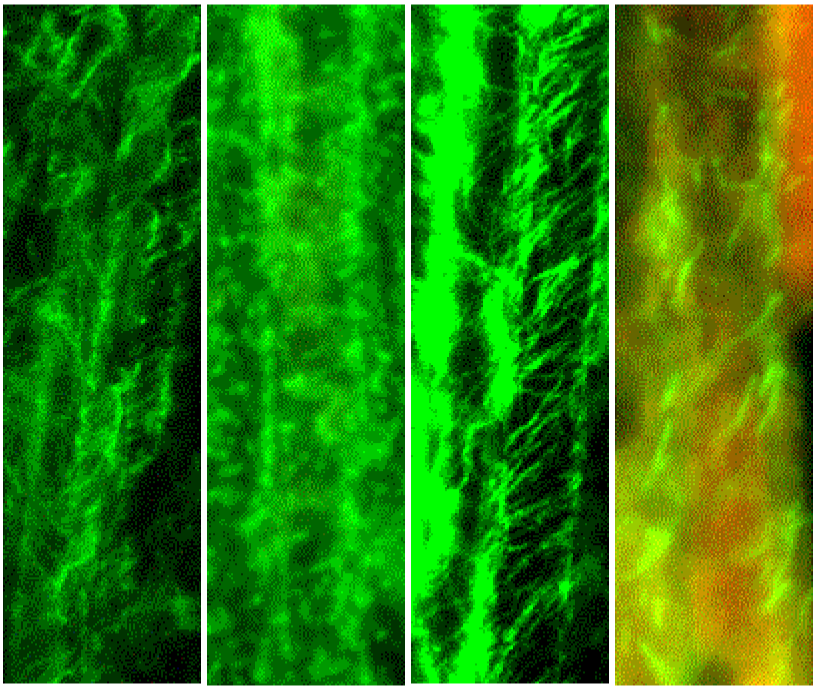

Figure 3: The visualization of the microtubule cytoskeleton in cells of the differentiation zone of wheat seedling roots. A, control (23 °C); B, + oryzalin (10 μM) at 23 °C; C, cold treatment (3 °C, 7 days); D, cold treatment + oryzalin (10 μM).

Table 1: The influence of oryzalin on the percentage of polymerized microtubule at high and low temperatures.

	23 ± 2 °C	3 ± 1 °C
- Oryzalin	100	100
+ Oryzalin	5	28

Chlorpromazin caused a 27 % increase of the electrolyte exosmosis from the root tissue (Figure 1A) and the activity of cell wall lectins had almost doubled (Figure 2A) while the activity of the soluble lectins (Figure 4A) and the soluble protein (Figure 5A) decreased by 77 and 38 %, respectively. Similar changes could be demonstrated in media with and without Ca^{2+} under the influence of oryzalin which increased the activity of cell wall lectins (Figure 2B) whereas the activity of soluble lectins was decreased (Figure 4B).
No differences in the influence of chlorpromazin on lectin activity could be found in media with and without Ca^{2+} while higher electrolyte exosmosis from the tissue and a more pronounced decrease in soluble protein content were observed in the Ca^{2+} treatment.
Combined application of chlorpromazin and oryzalin did not result in additive effects. In this case all tested parameters yielded similar data as with chlorpromazin (Figures 1A and B, 2A and B, 4A and B, 5A and B).
Oryzalin did not influence electrolyte exosmosis after cold adaptation (Figure 1C) while under this condition microtubule aggregation was more pronounced (Figure 3C and D).
In the cold treatments oryzalin reduced the number of preparations exhibiting polymerized microtubules to a considerably lesser degree than at 23 °C (Table 1) whereas the other parameters responded as at 23 °C. The cold adaptation caused a smaller change of plasmalemma permeability (Figure 1C) and the activity of cell wall lectins (Figure 2C) under the influence of chlorpromazin whereas the reduction of the soluble lectin activity (Figure 4C) and the soluble protein content (Figure 5C) was considerably stronger.
After the application of exogenous calcium an increase in electrolyte exosmosis of 14 % (Figure 1D) and a doubling of cell wall lectin activity (Figure 2D) was noted in the oryzalin treatment while calcium did not affect the influence of oryzalin on the content of soluble protein (Figure 5C and D). The effect of chlorpromazin on the permeability of the plasmalemma (Figure 1D) and the activity of cell wall lectins (Figure 2D) was reduced in cold adapted (hardened) roots in both the plus and minus calcium treatment while the reduction of the soluble lectin activity (Figure 4D) and the soluble protein content (Figure 5D) due to the chlorpromazin treatment were the same at 23 and 3 °C. In cold adapted roots no additional effects on the tested parameters could be seen if chlorpromazin and oryzalin were applied in combination.

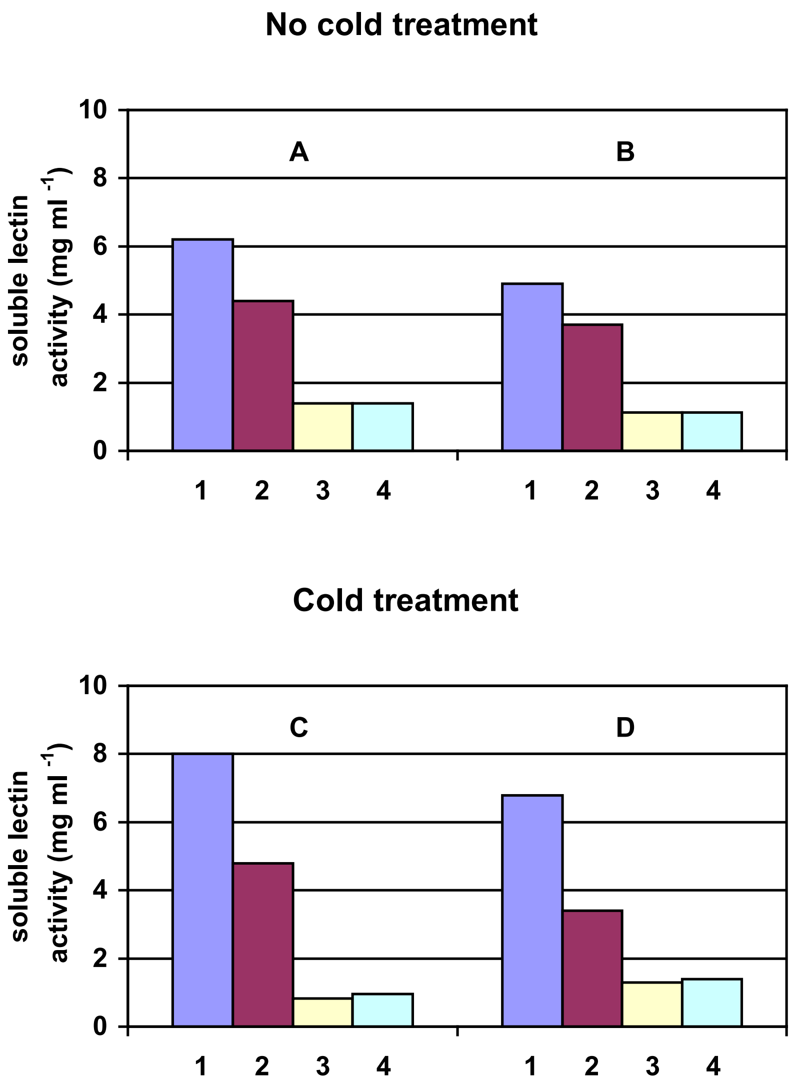

Figure 4: Soluble lectin activity in roots of wheat seedlings as influenced by oryzalin and chlorpromazin: A, C, - Ca^{2+}; B, D, + Ca^{2+}; 1, control; 2, + oryzalin (10 µM); 3, + chlorpromazin (250 µM); 4, + oryzalin + chlorpromazin.

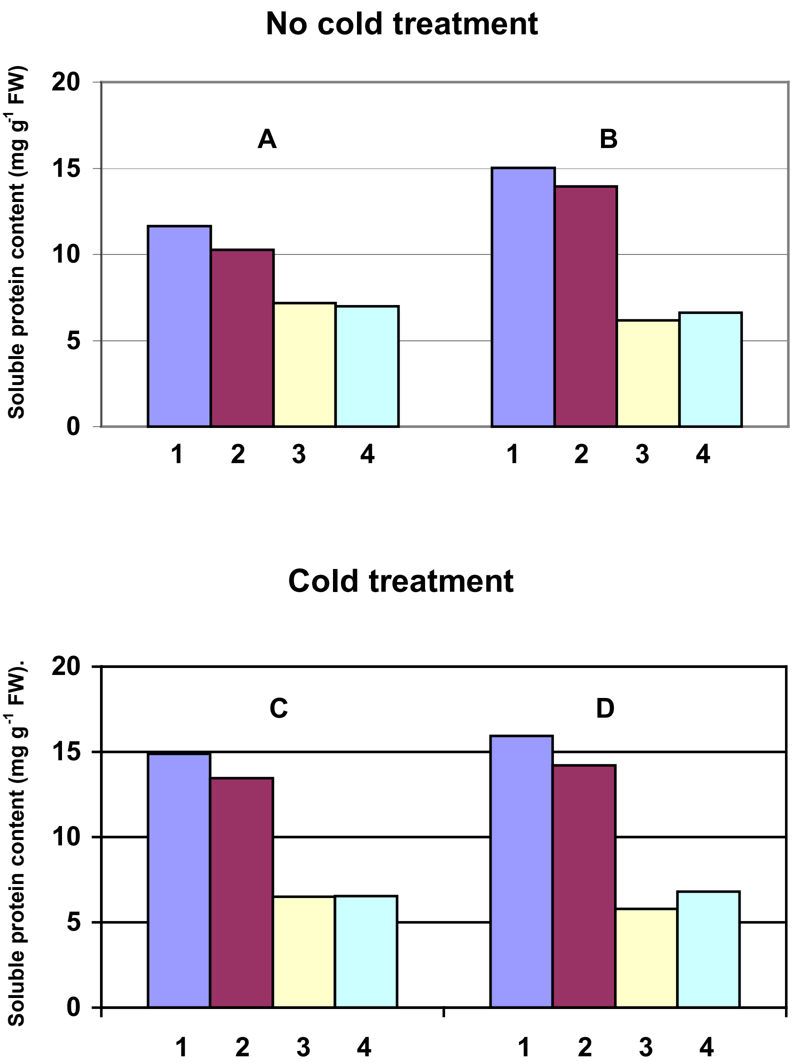

Figure 5: Soluble protein level in roots of wheat seedlings as influenced by oryzalin and chlorpromazin: A, C, - Ca^{2+}; B, D, + Ca^{2+}; 1, control; 2, + oryzalin (10 µM); 3, + chlorpromazin (250 µM); 4, + oryzalin + chlorpromazin.

Discussion

Our data show that at optimal temperatures (23 °C) the structure of microtubules was altered under the influence of oryzalin. Binding to tubulin, oryzalin inhibits the polymerization, fosters the disintegration of existing and impedes the formation of new microtubules (Morejohn et al., 1988) – perhaps because of the reduction of the tubulin content (Giani et al., 1998). Apparrently, the microtubule disintegration observed in our studies enhanceded the changes in cell reactions, i.e. the increase in plasmalemma permeability and cell wall lectin activity as well as the reduction of soluble lectin activity and soluble protein content.

On the other hand, cold adaptation enhanced the structural stability of the tubulin cytoskeleton thereby counteracting the effect of oryzalin. This can probably be related to the synthesis of new microtubule association proteins (MAPs; Cyr and Palevitz, 1989) and the improvement of the interaction of cortical microtubules with the plasmalemma (Baluska et al., 1993) which resulted in the formation of cold stable membrane-cytoskeleton complexes and a reduction of plasmalemma permeability. Hence, the electrolyte exosmosis observed under the influence of oryzalin is reduced in cold adapted (hardened) roots of wheat seedlings. In an earlier report, we demonstrated the maintenance of free Ca^{2+} in oryzalin treated seedling leaves at optimal temperatures (Asafova et al., 1999). In the investigations described here the same effect of oryzalin on the permeability of the plasmalemma and the activity of cell wall bound and soluble lectins in seedling roots incubated with and without Ca^{2+} at optimal temperature conditions could be demonstrated. This was the basis for the assumption that exogenous Ca^{2+} had no influence on cytosolic Ca^{2+} in root cells and that the disorganization of the tubulin cytoskeleton was not enhanced by an increase of free Ca^{2+}. Keifer et al. (1992) deny the influence of µM concentrations of oryzalin on the Ca^{2+} status of the cell. Yet, there are other results: Oryzalin increased the activity of Ca^{2+} channels (Thion et al., 1996) and in our experiments the reduction of the soluble protein content due to the oryzalin treatment was the same with and without Ca^{2+} amendment. It is also known that protease activities are under the control of Ca^{2+} ions (Tarchevsky, 1993) which supportes our assumption as to the maintenance of the Ca^{2+} status of the cells. This interpretation is in accordance with the results of Sonobe (1990) who could not find disorganisation of microtubules in a medium containing 1 mM $CaCl_2$. As was outlined earlier, the disintegration of microtubules is related to the increase of Ca^{2+} in the cells (Schliwa et al., 1981). Cyr (1991) was able to demonstrate that 1 mM Ca^{2+} concentration in the cytosol of carrot protoplasts destabilized their microtubules.

The increased oryzalin sensitivity of the root cell plasmalemma induced by cold adaptation and Ca^{2+} can be interpreted as the consequence of the time lag for the formation cold resistant microtubule poulations. Further, changes in the phosphorylation status of proteins which are linked to the microtubules or a destabilizing effect of the Ca^{2+}-calmodulin complex on cortical microtubules may have caused the observed effects (Fisher et al., 1996).

In order to test this assumption we used chlororomazin which inhibits the formation of the Ca^{2+}-calmodulin complex. Under optimal temperature conditions this inhibitor diminished the activty of soluble lectins and, simultaneously, increased that of cell wall bound lectins in roots of seedlings grown with and without Ca^{2+}. According to modern views calmodulin stabilizes cortical microtubules (Fisher et al., 1996). Perhaps, chlorpromazin is more effective in microtubule disorganization than oryzalin. This may explain the observation that no effect of oryzalin could be seen in chlorpromazin treated tissue. Chlorpromazin does not only influence the organization of microtubule due to the destruction of the Ca^{2+}-calmodulin complex but may also function as a membraneotropic agent. This is confirmed by our results which showed that electrolyte exosmosis was increased to a greater extent in chlorpromazin treated roots than under the influence of oryzalin and that applied Ca^{2+} enhanced this effect. In the literature it was discussed that one reason for the destabilizing effect of the Ca^{2+}-calmodulin complex is its capability to destroy the contacts between microtubules and the plasma membrane (Fisher et al., 1996). Cold treatment diminished some of the chlorpromazin effects in both the plus and minus Ca^{2+} treatments. These phenomena may be linked to the formation of Ca^{2+}-resistant microtubule populations as well as to the capability of exogenous Ca^{2+} to improve cold hardening. This could have been brought about through the aggregation of membrane components by Ca^{2+} and the improvement of plasmalemma elasticity due to the formation of Ca^{2+}-bridges between phospholipids and proteins (Issabekov and Krassavcev, 1989).

It is assumed that the changes of the stability of the tubulin cytoskeleton and its interactions with the plasmalemma is one of the links in the chain of signaling systems. These systems are regulated by exogenous calcium and low temperature which govern the biochemical and physiological responses of the plant. Therefore, it can be concluded that the cytoskeleton functions as a mediator between exogenous signals and cell reactions in plants.

References

Asafova, E., L. Khokhlova, I. Volovnik, R. Chestnova, E. Makarova, K.-H. Neumann and Ch. Lein (1999): Der Einfluss des Oryzalin, eines Inhibitors der Tubulinpolymerization bei unterschiedlichem Calcium- und Temperaturniveau bei Winterweizenkeimpflanzen. In: Die Vielfalt der Zusammenarbeit. Zum zehnjährigen Jubiläum des Vertrags über die Zusammenarbeit zwischen der Staatlichen Universität Kasan und der Justus-Liebig-Universität Giessen. Kasan Unipress, pp 366-377.

Baluska, F., J.S. Parker, P.W. Barlow (1993): The microtubular cytoskeleton in cells of cold-treated roots of maize (Zea mays L.) shows tissue-specific responses. Protoplasma 172, 84-96.

Baluska, F., S. Vitha, P.W. Barlow and D. Volkmann (1997): Rearrangements of F-actin arrays in growing cells of intact maize root apex tissues: A major developmental switch occurs in the postmitotic transition region. Europ.J.of Cell Biol. 72, 113-121.

Cyr, R.J. (1991): Calcium/calmodulin affects microtubule stability in lysed protoplasts. J. Cell Sci. 100, 311-317.

Cyr, R.J. and B.A. Palevitz (1989): Microtubule-binding proteins from carrot. 1. Initial characterization and microtubule bundling. Planta 177, 245-260.

Fisher, D.D., S. Gilroy and R.J. Cyr (1996): Evidence for opposing effects of calmodulin on cortical microtubules. Plant Physiol. 112, 1079-1087.

Fulton A. (1987): Cytoskelett. Architektur und Choreographie. Mir publishers, Moscow , 117 pages.

Giani, S., X. Qin, F. Faora, D.Brevirio (1998): Oryzalin and protein levels in rice. Planta 205, 334-341.

Issabekov, B.N. and O.A. Krassavcev(1989): Osmotische Eigenschaften der frostresistenten Protoplasten. Russ.J.of Plant Physiol. 36/2, 372-386.

Keifer, A.Q., D.A. Callacham and P. Hepler (1992): Inhibitors of cell division and protoplasmic streaming fail to cause a defeatable effect on intracellular calcium levels in stamen-hair cells of Tradescantia virginiana L. Planta 186, 361-366.

Kumar, A., L. Bender, B. Pauler, K.-H. Neumann, H. Senger and C. Jeske (1983): Ultrastructural and biochemical development of the photosynthetic apparatus during callus induction in carrot root explants. Plant Cell Tiss. Org. Cult. 2, 161-177.

Lloyd, C.W., B.K. Drobak, S.K. Dova and C.J. Staiger (1996): Interaction between the plasmamembrane and the cytoskeleton in plants. In: M. Smallwood, J.P. Knpx, D.J. Bowles (eds.) Membranes: Spezialized Functions in Plants. BIOS Scientific Publishers(UK) pp 1-20.

Morejohn, L.C., T.E. Bureau, J. Mole-Bajer, A.S. Bajer and D.E. Fosket (1987): Oryzalin, a dinitroaniline herbicide, binds to plant tubulin and inhibits microtubule polymerization in vitro. Planta 172, 252-264.

Nick, P. (1998): Signaling to the microtubular cytoskeleton in plants. Intern. Review of Cytology 184, 33-79.

Schliwa, M., U. Euteneuer, J. Ch. Bulinski and J.G. Izant (1981): Calcium lability of cytoplasmic microtubules and its modulation by microtubule-associated proteins. Cell Biology 78/2, 1037-1041.

Sonobe, S. (1990): ATP-dependent depolimerization of cortical microtubules by an extract in tobacco BY-2 cells. Plant Cell Physiol. 31, 1147-1153.

Sonobe, S. and S. Takahashi (1994): Association of microtubules with the plasma membrane of tobacco BY-2 cells in vitro. Plant Cell Physiol. 35/3, 451-460.

Tarchevsky, I.A. (1993): Katabolism und Stress bei Pflanzen. Nauka publishers, Moscow, 80 pages.

Thion, L., C. Mazars, P. Thuleau, A. Graziana, M. Rossignol, M. Moreau and R. Ranjeva (1996): Activation of plasma membrane voltage-dependent calcium-permeable channels by disruption of microtubules in carrot cells. FEBS Letters 393, 13-18.

Timofeeva, O.A., L.P. Khokhlova, T.V. Trifiniva, N.E. Belyaeva, J.J. Chulkova (1999): Effect of Cytoskeleton Modifiert on the Lectin Activity during Plant Acclimation to low Temperatures and under ABA Treatment. Russian J. of Plant Physiology 46/2, 147-151.

Vasiljev, A.E. (1996): Vergleichende struktur-funktionelle Charakteristik des Cytoskeletts von Tieren und höheren Pflanzen. J. Allgemeine Biologie 57/3, 293-325.

Williamson, R.E. (1987): Calcium and the plant cytoskeleton. Plant Cell Environ. 7, 431-440.

Ayurvedic Medicines: Some Potential Plants for Medicine from India

Ashwani Kumar

Energy Plantation Demonstration project and Biotechnology Center,

Department of Botany, University of Rajasthan, Jaipur, 302004 (Rajasthan) India.

e-mail: msku4@hotmail.com

Abstract

With the changing pattern of life style most of the diseases are now becoming lifestyle diseases. The traditional systems of medicine based on ancient cultures are primarily concerned with building the body strength which can help in healing the ailments and these systems rely largely on the nature cure. The Ayurvedic system has described a large number of such medicines based on plants or plant product and the determination of their morphological and pharmacological or pharmacognostical characters can provide a better understanding of their active principles and mode of action.

Introduction

Contribution of the traditional medicine to human health in the 21st Century is of paramount importance. A meeting of the International Forum on Traditional Medicine held recently (1999) at theToyama Medical and Pharmaceutical University, Toyama, Japan, reviewed the potential of traditional medicines. WHO acting director Xhang emphasized that with the changing pattern of life style most of the diseases are now becoming life style diseases. Natural medicines improve the inner strength of the body. The use of traditional medical systems has attracted so much attention that an International Health Center has been opened in July in the Toyama prefecture (Province)

Some of the oldest traditional medical systems include Chinese, Ayurvedic, Unani, Japanese and recently added homeopathy and chiropathy that is also around 200 years old. The use of traditional medicine includes (i) medication by use of medicinal plant, minerals, animal material and (ii) non medication: acupuncture and yoga.

Complementary medication includes acupuncture, herbal treatment, manual, spiritual and dietary treatments.

Toyama hospital utilizes vast amount of Chinese, Japanese and Ayurvedic medicine. Detailed studies in the areas of pharmacognosy and pharmacology are under progress (Annual report, TMPU, Toyama). Besides this the Research Center for Ethnomedicines with its Museum of Materia Medica is one of its own kind in the world under Professor Watanabe. Dr. Komatsu provides a wealth of information for all scientists engaged in the field all over the world. This includes identification, molecular characterization at DNA sequence level, chemical characterization, biotransformations and studies on effects on microorganisms to direct application in the hospital. To give an entire description will be attempted in another paper. Here a brief attempt is made to identify common goals of research in India and Japan, with an objective to attract attention of workers to the great potential that the vast bio-diversity of the Indian subcontinent and the wealth of Ayurvedic literature has to offer for future development of traditional medicines. However detailed future investigations are needed in this area to exploit the unexplored or poorly explored plant materials.

These traditional medicines have found practical application at clinical level in TMPU and over hundred cases of fissure have been cured in the hospital using a special thread prepared from latex of Euphorbia spp., thor of India (Euphorbia sp) , haldi powder (*Curcuma longa)* and some herbal ingredients. The *Euphorbia* sp is a plant of the desertic region of India and different parts of the world. A large number of energy yielding desertic plants of India used in the Ayurvedic system have great potential as Ayurvedic medicine . Negative environmental effects of current agricultural practices, such as emission of greenhouse gases, nutrient leaching, decreased soil fertility, and erosion, may be reduced when traditional annual food crops are replaced by dedicated perennial energy crops and medicinal plants. As they are able to grow and produce valuable products under dessert conditions they have great potential for covering the global desert areas into green belts leading to environmental improvement on one hand and providing valuable Ayurvedic crude drugs in addition to supplementing the bio-energy resources as renewable fuels. However detailed studies on their pharmacognostical characterization and determination of chemical products obtained from them are lacking. Some of the investigations indicated their potential use in Human immuno deficiency (HIV) diseases (Hattori et al., 1995). Such bio-energy plants have not been explored in depth. Here an attempt shall be made to provide a brief outlook of the Indian scene and highlight some of the work being carried out at our place in Rajasthan along with the possible impact assessment for desertic plants for future research strategies.
Among the desert plants the value of *Aloe vera* (L.) was recognized more than 3000 years ago when the Egyptian and Greek civilizations used its extract for skin burns, cuts and wounds on the skin surface and found that it had a wonderful healing effects on the skin. It is claimed that even 3rd degree burns can be cured and healed by *Aloe vera*. The chemical compounds like Aloein, resins and a mixture of polysaccharides containing pectic acid are present. Modern investigations indicate that extracts of *Aloe vera* act on the dead epithelial cells of the skin, aiding their removal from the surface and stimulating

the growth of new cells. Thus Aloe is a great gift of traditional medicine for protecting the smooth skin of human beings especially when radiation damage has assumed an alarming situation due to stratospheric ozone depletion. Fresh juice of leaves are also used in liver and spleen troubles and also for eye troubles, found useful in X-ray burns, dermatitis, coetaneous and other skin disorders.

In India, Egypt and Sudan around 70 percent of the rural people use traditional medicine. Similar situation exists in a large number of developing countries. In India and China 60 percent of the people affected with cholera and malaria are treated with herbal medicines. In these countries the market for traditional medicines is US $ 500 million while Western type medicine account for only 300 million US $. In Singapore 50 percent and in Australia 60 percent of population uses alternative medicine. Around 17,000 herbal products are registered in these countries. In Belgium 40 percent contemporary but 84 percent home medicines and 74 percent acupuncture medicine is utilized. In France 50 percent of the people take advantage of complementary medicine. In Germany 10,000 to 13, 000 alternative medical practitioners are thriving well and 75 percent of them utilize complementary medicines. 77 percent of pain clinics utilize acupuncture. In UK 90 percent of the complementary medical practitioners utilize osteopathy and acupuncture. In US where in 1990 only 30 percent of the people were utilizing complementary medicines, it grew to 40 percent in 1997.

Ayurvedic system of Medicine

Ayurveda is an offshoot of Atharva veda written over 3000 thousand years ago. The Charak and Sushruta describe a large number of crude drugs and a large part of them has origin to plants. However though some part of it has been translated from Sanskrit to Japanese and the Japan Society of Ayurveda under Professor Dr. Namba is very active in this field. But many of the crude drugs described remain to be identified to its plant source in botanical terms and the Institute of Traditional Medicine is the prime center for understanding the nature and morphology of crude drugs of Ayurvedic origin and their identification to the plant level. The personal communication with Professor Watanabe and Dr. Komatsu during my stay at Wakan Yaku as visiting Professor has contributed to the stimulation of such studies back home and some of the important findings are presented here. During my stay here I have worked on Nepalese crude drugs with support and guidance from Dr. Komatsu and other members of this institute.

The basic philosophy of Ayurveda considers that man is an inseparable part of the universe. The human body, mind and spirit continuum is an integral whole and the individual is also linked to the family, society, environment and ultimately the universe. The definition of health is that " It is state of complete psychosomatic equilibrium. It does not mean only absence of diseases but a state in which the mind, senses and spirit are pleasant and active". That agrees with the definition of WHO "Health is a state of complete physical, mental and social well being and not merely the absence of disease or infirmity"

India with its varied climate, soils and agro-ecology possesses an immense plant diversity, with over 15,000 species of higher plants. Both our Indian civilization as well as our diverse tribal heritage have gone a long way in conserving the wild weedy species, native land races and primitive cultivars (Fig. 1). The Indian gene center is endowed with rich flora, especially with regard to several less known yet economically important plants, ca. 160 cultivar species of economic plants, plus 56 species of lesser known cultivated food plants. Further there are ca. 320 species of wild and weedy economic types (Paroda, 1979; Arora and Nayar, 1984 ; Kumar, 1998).

The unutilized and underutilized resources

Out of 2,50,000 plant species only 10,000 or so have been exploited during the course of human civilization. A large number of hydrocarbon yielding plants are able to grow under semi arid and arid conditions and they also produce valuable hydrocarbons (up to 30 percent of dry matter) which could be converted into petroleum-like substances and used as fossil fuel substitute. They are rich in triterpenoids which are constituent to important drugs against HIV.

The potential plants

Certain potential plants were selected and attempts were made to develop agro-technology for their large scale cultivation (Kumar et al, 1995, Kumar, 1998, Kumar, 2000). A 50 ha bio-energy and medicinal plants cultivation demonstration center has been established on the campus of the University of Rajasthan to conduct the experiments on large scale cultivation of selected plants with the objective of developing optimal conditions for their growth and productivity, besides conserving the bio-diversity. Plantation of laticiferous plants and desert plants can be carried out, it could also lead to reclamation of marginal land that has already been abandoned in developed as well as developing countries. India alone has over 144 million hectare of marginal land which is about half of the total geographical area of the country. Touched only marginally by the green revolution, Africa suffers not only a dramatic nutritional problem but also an equally serious and inter linked problem of energy. Increasing scarcity of fuelwood, desertification, lack of water, food and medicines, excessive urbanization are all closely interdependent and rich biodiversity in developing countries has remained unutilized and underutilized for want of proper investigations.

There are surely opportunities for biomass of the medicinal plants in the south as well as in the north in wet climates and in dry ones but they will respond to very different schemes and strategies. There is not going to be a single unique recipe, rather multiplicity of solutions depending on climate, soil, availability of land, traditions as well as social and economic conditions. Technological improvements should lower production costs but they are unlikely to obtain significantly higher yields, as chemical and energy inputs must be reduced. The transformation of biomass into useful energy products and medicinal compounds may however involve onsite industrial operations that could absorb at least part of the surplus man power.

As far as research is concerned we are all aware of the important progress being made in agricultural biotechnology. Genetic engineering for example is increasingly applied to crop plants for improving resistance to pests and diseases and for providing more favorable crop composition. There is a whole universe of possibilities in the use of advanced biotechnology to improve plants and processes. The natural medicine from plants has enormous possibilities for new and more effective means for curing the modern day ailments.

Natural resource

Total land area of Rajasthan is 3,42,239 sq km out of which 45.25 percent is characterized as wasteland. Large portions of this land were productive at a given time and due to man made deforestation, cattle pressure, water and wind based soil erosion, improper water management, they have turned out to be wastelands. (Kotia and Kumar, 2001a). A detailed survey on the weeds on wastelands yielded valuable data about the first colonizers. Out of the total weeds around 50 having important medicinal values while others produce related compounds. These regions are rich in bio-diversity and weeds were collected from different regions of the developing wastelands. (Kotia and Kumar, 2001b) .
Some of the medicinally important plants of Rajasthan are listed by Ajanta and Kumar, (2001a) They include species listed in table 1:

Table1: List of Medicinal plants of Rajasthan.

Plant species:	Local name
1. *Asparagus racemosus*	Satavari
2. *Chlorophytum arundinaceum*	Safed musli
3. *Curculigo orchioides*	Kali Musali
4. *Solanum surattense*	Kantkari
5. *Boerhaavia diffusa*	Santhi,
6. *Hamidesmus indicus*	Anantmool
7. *Sida cordifolia*	Bala
8. *Holarrhena antidysenterica*	Indrajo
9. *Curcuma aromatica*	Vanhaldi
10. *Oroxylum indicum*	Shyonaka
11. *Balanites aegyptiaca*	Hingot
12. *Withania somnifera*	Ashwagandha
13. *Aegle marmelos*	Bael
14. *Cassia fistula*	Amaltas
15. *Gymnema sysvestre*	Gudmar
16. *Terminalia arjuna*	Arjuna
17. *Butea monosperma*	Palas

18. *Soymida febrifuga*	Rohan
19. *Woodfordia fruticosa*	Dhavri
20. *Tribulus terrestris*	Gokhru
21. *Pedalium murex*	Badagokhru
22. *Vitex negundo*	Negad
23. *Dyerophytum indicum*	Chhitral
24. *Plumbago zeylanicum*	Chitrak
25. *Plantago ovata*	Isabgol
26. *Colocynthes vulgaris*	Indrayan
27. *Adhathoda vasica*	Ardusta
28. *Allangium salvifolium*	Aankol
29. *Caesalpinnia bonducella*	Tas
30. *Jatropha curcas*	Ratanjot
31. *Eclipta alba*	Bhringraj
32. *Aloe barbadensis*	Gwarpatha
33. *Mucuna prutita*	Konch
34. *Terminalia bellerica*	Baheda
35. *Tamarindus indica*	Imli
36. *Azadirachta indica*	Neem
37. *Achyranthes aspera*	Aandhijhara
38. *Barleria cacrulea*	Bajrandantis
39. *Barleria cristata* .	Badradantip
40. *Barleria prinoitis*	Bajradantip.
41. *Ocimum americanum*	Bapchii
42. *Centella asiatica*	Brahmibuti
43. *Datura metel.*	Dhatura
44. *Convolvulus arvensis*	Haranpadi
45. *Evolvulus alsinoides*	Shankhpushpi
46. *Cassia occidentalis*	Kasaundi
47. *Urginea indica*	Kolikanda
48. *Andrographis paniculata*	Kalmegh
49. *Helicteres ispara*	Marorphali
50. *Tinospora cordifolia*	Nimgiloy

Calotropis procera (Ait.) R.Br. (Akanda, Alarka, Aak) : The plant is one of the important numbers of traditional herbal medicine in every home of India. Traditionally the leaves of aak are warmed and tied around any body organ in pain. It is practically useful in backache and in joint pains. Warm leaves also relieve from stomach ache if tied around. Inhalation of burnt leaf cures headache. The traditional folk healers use the milky latex of aak for several ailments. Leaf latex if applied on fresh cut, stops bleeding immediately. Recent investigations have found that the alkaloids calotropin, calotaxein and uskerin are stimulant to the heart. Flowers and roots are used in Ayurvedic medicine. The plant is anthelmintic, the ashes act as an expectorant. The leaves are applied hot to the abdomen to cure the pain inside. The flower is tonic, antisialagogue, used as appetizer and against stomach ache, and cures piles and asthma. Flowers are believed to have detergent properties so they are given in cholera. The fresh roots are used as a toothbrush and are considered by pathans to cure toothache.. Alarka is an alternative tonic and diaphoretic, in large dose emetic. Root bark is useful for treating chronic cases of dyspepsia, flatulence, constipation, loss of appetite, indigestion and mucus in stools. Leaves are used against guinea worms. Flowers are useful in asthma. Seed oil is geriatric and tonic. Green copra is given in asthma. Plant is used in spleen complaints, rheumatism, epilepsy, hemiplegia, sores, and smallpox and protracted labor.

Calotropis gigantea R.Br. (Arka) : Arka is purgative, anthelmintic alexipharmic,; cures leprosy, ulcers, leucoderma, tumors, piles, diseases of spleen, liver and abdomen. Juice is anthelmintic and laxative; cures piles and kapha. Dried and powdered plant is taken with milk and acts as a good tonic. Action is similar to Digitalis on the heart. Root bark and juice have emetic, diaphoretic, alternative and purgative properties. It is used in dysentery and as a substitute for Ipecauantha. It is regarded as a great remedy in syphilitic afflictions and is called “Vegetable mercury”. In intermittent fevers it is used as antiperiodic and diaphoretic. It cures asthma and syphlis. In form of paste it is applied to elephantiasis. Tincture of leaves is used in intermittent fevers. Latex is bitter, heating, oleagenous and irritant, used in combination with *Euphrobia neerifolia* as purgative. Flowers are sweet, bitter, digestive, tonic, stomachic, anthelmintic, analgesic, astringent; cure inflamations, tumours, kapha and are good in ascites.

Jatropha curcas Linn. (Vyagrairanda) : Juice of Vyagrairanda is a well known purgative and is useful in whitlow, convulsions, syphilis, neuralgia, dropsy, anasarca, pleurisy and pneumonia. Root bark is applied externally in rheumatism and is used in sores. Leaves are galactagogue, rubefacient, suppurative, insecticidal and are used in foul ulcers, tumors and scabies, given internally in jaundice. Leaves are locally applied to breasts to increase secretion of milk. Leaves warmed and rubbed with castor oil and applied to boils and abscesses have supportive effect. Decoction of leaves is against diarrhoea, useful in stomach-ache and cough and also used for gargle to strengthen gums. Fresh stems are used as toothbrush. Fresh viscid juice flowing from stem is employed to arrest bleeding or hemorrhage from wounds. Stem bark is used for wounds of animal bites. Fruits and seeds are anthelmintic, useful in chronic dysentery, urinary discharges, abdominal complaints, anaemia, biliousness, fistula, and diseases of heart. Seeds are acro-nacrotic, poisonous to human beings and cattle and used against warts and cancers and also to promote hair growth. Seeds and oil are purgative, more drastic than castor oil. Wood causes dermatitis. Drug is bitter, acrid, astringent and anthelmintic. It serves to cleanse the entire system through its purgative property. It is useful in chronic dysentery, thirst, abdominal complaints, biliousness, anemia, fistula, ulcer, and diseases of the heart and skin.

Croton tiglium Linn. (Jamaalagotta, Jayapala) : Jayapala seeds and oil are drastic purgative, diaphoretic, vasicant, vermifuge irritant, rubefaceint and cathartic. Its action is prompt. Croton oil when rubbed on skin acts as a rubefaceint and counter-irritant and vesicant. When administered internally it operates as a powerful hydrogue cathartic. It is found to be very useful in ascites, anascara, cold, cough, fever, asthma, constipation, calculus, dropsy and enlargement of abdominal viscera. It is given only when a drastic purgative is required as in dropsy and cerebral affections like convulsions, insanity and other fevers, attended with high blood pressure. Wood is diaphoretic in small doses and purgative and emetic in large doses.

Euphorbia hirta Linn. (Dudhi, Cara) : Cara is demulcent, antispasmodic, anti-asthmatic pectoral, anthelmintic and local parasiticide. Plant is chiefly used in the affections of childhood, in worms, bowel complaints and cough, in postnatal complaints, failure of lactation, breast pain. Extract of plant has depressant action and action on cardiovascular system, a sedative effect on mucous membranes of the respiratory and urino-genitory tract. Juice of plant is given in dysentery and colic, and milk applied to destroy warts. Plant alkaloid is effective in respiratory system and produces dilation of bronchi. Decoction of plant is used in bronchial affections and asthma. Latex is vermifuge and used in diseases of urino-genitory tract and also in application for warts.

Euphorbia tirucalli Linn. (Vajraduhu, Satsala) :It is useful in biliousness, leucorrhoea, leprosy, dropsy, whooping asthma, enlargement of spleen, dyspepsia, jaundice, colic tumours, and stones in bladder. Milky juice is vesicant and rubifacient. In small doses a purgative but in large doses it is acrid, emetic and counter-irritant; application for warts, neuralgia, rheumatism, toothache, asthma, cough and earache. It is also a fish poison. Milky juice is applied to itch and scorpion bites. Decoction of tender branches and that of roots is administered in colic and gastralgia.

Anti-HIV agents among desert plants

Around 40million people are affected due to the Human Immuno-deficiency Virus globally. During the past decades, a large number of anti-viral screening experiments on medicinal plant extracts have been reported and have led to the selection of several extracts active towards herpes viruses. A promising result of a naturally occurring antiherpetic agent was given by n-docosanol (a natural 22 carbon saturated fatty alcohol) which is undergoing phase III clinical trials in patients. Clinical testing of the topical formulation, or systemic administration of drug suspensions has demonstrated a good therapeutic index, since high doses of n-docosanol do not elicit appreciable toxicity. The findings show that natural products are still potential sources in the search for new antiherpatic agents(Hattori et al., 1995,). Various plant extracts used in Ayurvedic medicine for inhibitory effects on HIV virus have been studied (Hattori et al., 1993 ; Kusumoto et al., 1995; Hattori, personal communication). A large number of such plants occur in semi-arid and arid climate of Rajasthan (Roy and Kumar, 1995) .

Acquired immunodeficiency syndrome (AIDS) , the great pandemic of the second half of the 20^{th} Century, is still a threatening disease world wide. Many research approaches are currently aimed at developing novel agents to arrest the replication of HIV through various targets. These may include the inhibition of reverse transcriptase (RT), protease (PR), membrane fusion and integrase. HIV PR enzyme has been demonstrated to play an essential role in viral replication (Meek et al., 1990). It is considered as potential target for anti-AIDS therapy, as the inhibition of this enzyme produces immature, non-infectious virions (Mous et al., 1988; Huff, 1991; Robins and Platter, 1993). A range of

HIV PR inhibitors have been designed and applied in clinical trials such as Sanqunavir, Ritonavir and Indinavir. However, the development of drug resistance by virus, irrespective of the target, remains as an overwhelming problem in AIDS chemotherapy (El Farrash et al. 1994). Thus there is great need to search for and develop new and different anti-HIV candidates from plants and natural products are of considerable importance.

In search for anti-HIV active agents from natural products, many attempts at screening traditional medicines have been made (Chang and Yeung, 1989; Otake et al., 1995; Wan et al., 1996). However Indian and other tropical region plants with their vast diversity, have not been investigated for their antiviral activity. Hussein et al. (1999) investigated forty eight methanol extracts from Sudanese plants which were screened for their inhibitory activity on viral replication. Nineteen extracts showed inhibitory effects on HIV–induced cytopathic effects (CPE) on MT-4 cells. The extracts were further screened against HIV-1 protease (PR) using an HPLC assay method. Of the tested extracts, the methanol extracts of the desert plants *Acacia nilotica* (bark and pods), *Euphorbia granulata* (leaves) , *Maytenus senegalensis* (stem-bark) and aqueous extracts of *A. nilotica* (pods) and *M. senegalensis* (stem-bark) showed considerable inhibitory effects against HIV-1 PR (Hussain et al., 1999) . Some of the plants from Sudan are common within the Indian dessert region of Rajasthan and generally they grow on the wastelands. They have potential use as bio-energy plantations (Kumar et al., 1995; Kumar, 1998). However a large number of them are used in the medicines of Ayurveda. They were also found effective against HIV-1. (Hussein et. al., 1999) . A list of potential plants of this region is given here in table 1. However these plants have not been studied in detail and there is need to study them for their medicinal properties including anti-HIV properties. Some of the active principles against anti-HIV are triterpenoids which are abundant in laticiferous plants of Rajasthan. Besides, *Ganoderma* sp is very frequently met in Rajasthan attacking trees. *Ganoderma lucidum* has been described to contain triterpenes which have inhibitory effects against HIV-1 protease (Min et al., 1998). Besides this, several other plants like *Abrus precatorius* L., Leguminosae (Chao-mei et al., 1998) , *Datura stramonium* L., *Balanites aegyptiaca* L. Delile etc. commonly found in Rajasthan show anti-HIV activity (Kawahata et al., 1996). In China, its seeds have been used as an insecticide and for skin diseases since ancient times .

A detailed survey of medicinally important plants has been carried out and important trees, shrubs and herbs have been listed and their characters studied in several publications from our laboratory. They included drugs for cure of urinary tract infection (Ajanta and Kumar, 2000b) anti-depressant herbal drugs (Ajanta and Kumar, 2000c), medicines for skin diseases (Shivani and Kumar, 2000), anticancer drugs (Sharma and Kumar, 2000); anti-diabetic drugs (Ritu and Kumar, 2000). Herbal drugs of Leguminosae from Rajasthan have been studied (Sapna and Kumar, 2000). Herbal crude drugs for anti-malaria (Anita and Kumar, 2001); anti-paralytic (Vandana and Kumar, 2001). Besides this, herbal crude drugs for cure of hepatic diseases (Santosh and Kumar, 2001) and diseases of the digestive system (Mamta and Kumar, 2001) have been studied for their characters and investigations on their morphological and pharmacognostical characterization are in progress.

Conclusion

The sustainable land utilization in the ecologically fragile climate of semi arid and arid regions has to be guided by the principal of optimal utilization of resources. It is a matter of great interest that a large number of plants of the arid and semi-arid regions of the world are effective as anti-HIV agents. They are also used in variety to herbal and traditional medicines as listed in this paper. Our previous work on their bio-energy production potential, if combined with their crude drug potential could yield bio-fuels on one hand and valuable crude drugs on the other. However a large number of tropical plants have not been studied in detail for their chemical constituents, pharmacological properties of the extracts, and their pharmacognostical characterization including DNA sequencing etc. If a joint collaboration could be established in this direction, valuable information could be generated with wide ranging practical applications. This could also provide alternative land use pattern for the rural poor thriving on marginal lands on one hand and help in eco-restoration on the other. The use of bio-energy plants in the herbal crude drugs has great potential and detailed investigations are planned with the help and cooperation of different agencies. This paper provided a brief outline of the work in the area for future suggestions and improvement.

Acknowldegement

I am deeply indebted to Professor Watanabe, Director of the Research Institute of Wakan Yaku for providing me an opportunity to work as visiting Professor at the Institute and Dr. Komatsu for constant encouragement support during my stay and work at the TMPU. Toyama.The keen interest and encouragement by Professor Dr. Neumann during the course of this work is gratefully acknowledged.

References

Arora, R.K. and E.R. Nayar, (1984): Wild relatives of crop plants of India. NBPGR. Science Monograph No. 9, New Delhi.

Byung-Sun, M., N. Nakamura and H. Miyashiro (1998): Triterpenes from the spores of *Ganoderma lucidum* and their inhibitory activity against HIV-protease. Chem. Pharm.Bull. 46 (10), 1607-1612.

Chang, R.S. and H.W. Young (1989): Inhibition of the growth of human immunodeficiency virus in vitro by crude extracts of Chinese medicinal herbs. Antiviral Res. 9, 163-176.

Chao-mei, M., N. Nakamura and M. Hattori (1998): Saponins and C-Glycosyl Flavones from the seeds of Abrus precatorius. Chem. Pharm. Bull. 46 (6), 982-987.

Choudhary, M. and A. Kumar (2001): Ayurvedic crude drugs for cure of diseases of the digestive system. Int. J. Mendel. 18, 27-28.

El-Farrash, M.A., M.J. Kuroda and T. Kitazaki, et. al. (1994): Generation and characterization of a human immunodeficiency virus type 1 (HIV-1) mutant to an HIV-protease inhibitor. J. Virol. 68:233-239.

Gupta R. and Kumar, A. (2000): Ayurvedic crude drugs as potential cure of diabetes. Int. J. Mendel. 17(3-4), 127-128.

Hattori, M., I.T. Kusumoto, M. Soga and T. Namba (1993): Screening of various Ayurvedic medicines for their inhibitory activities on the reverse transcriptase and Identification of arecatanins, an embelin, as major inhibitory substances from Areca catechu and Embelia ribes. J. Med. Pharm. Soc. Wakan-Yaku 10, 141-148.

Hattori, M., T. Nabakayashi and Y. Lim et al. (1995): Inhibitory effects of various Ayurvedic and Panamian medicinal plants on the infection of Herpes simplex virus 1 in vitro and in vivo. Phythother. Res. 9, 270-276.

Huff, J.R. (1991): HIV-1 protease: A novel chemotherapeutic target for AIDS. J. Med. Chem. 34, 2305-2314.

Hussein, G., H. Miyashiva, N. Nakamura, M. Hattori et al. (1999): Inhibitory effects of Sudanese plant extract on HIV-1 replication and HIV-1 Protease. Phytotherapy Res. 13, 31-36.

J.R.Goodin and D.K. Nottington (eds.). International Center for Arid and Semi-arid land studies, Lubbock, Texas, pp. 261-281.

Kawahata, ,T., T. Otake, H. Mori, M. Morimoto, N. Ueba, I.T. Kusumoto, S. El-Mekkawy, M.Hattori, T. Namba (1995): J. Trad. Med. 13, 59-65.

Kotia, A. and A. Kumar (2001b). Some of the common weeds of medicinal value from Rajasthan . Int. J. Mendel. 18, 17-20.

Kotia, A. and A. Kumar (2001a): Characterization of weeds on wastelands and their role in eco-development . Int. J. Mendel. 18, 7-8.

Kumar, A. (1998): Biomass energy crops of semi-arid regions of India and their energy potential. In: Biomass for Energy and Industry (H. Kopetz et al., eds.) CARMEN, Germany. pp 345 – 348.

Kumar, A. , S. Johari, and S. Roy (1995): Production and improvement of bioenergy sources. J. Indian Bot. Soc. 74A, 233-244.

Kumar, A. (2000): Hydrocarbon yielding plants and future prospects: Biotechnological approach. In: Plant Biotechnology (P.C. Trivedi, ed.) Panima. New Delhi. pp 194-212.

Kusumoto, T. Nakabayashi H. Kida et al. (1995): Screening of various plant extracts used in Ayurvedic medicine for inhibitory effects on human immunodeficiency virus type 1 (HIV-1) protease. Phytother. Res. 9, 180-184.

Meek, T.D., D.M. Lambert, B.W. Metcalf, S.R. Petterway and G.B. Dreyer (1990): HIV-1 protease as target for potential anti-AIDS drugs. In: Design of Anti-AIDS Drugs. E. DeClerckq,ed. Elsevier Science Publishers B.V. , The Netherlands, pp. 225-256.

Mishra, A. and A. Kumar (2001b): Studies on Ayurvedic crude drugs for the cure of urinary tract stones. Int. J. Mendel 18 (1-2), 41-42.

Mishra, A. and A. Kumar (2001a): Medicinally important trees of Rajasthan. Int. J. Mendel. 18, 37-38.

Mishra, A. and Ashwani Kumar (2001c): Searching for anti-depressant crude drugs in ayurvedic system of medicine. Int. J. Mendel. 17 (3-4), 91-92.

Mous, J., E.P. Heimer and S.F.J. Le Grice (1988): Processing protease and reverse transcriptase from human immunodeficiency virus type 1 polyprotein in Escherischia coli. J. Virol. 62, 1433-1444.

Otake, T., H. Mori, M. Morimoto et al. (1995): Screening of Indonesian plant extracts for anti-human immunodeficiency virus type 1 (HIV-1) activity. Phytother. Res. 9, 6-10 .

Paroda, R.S. (1979): Plant resources of Indian arid zones for industrial uses. In: Arid land Plant resources.

Robbins, T. and J. Platter, (1993): HIV protease inhibitors, their anti-HIV activity and potential role in treatment. J. AIDS. 6, 162-170..

Roy, S. and A. Kumar (1995): Biodiversity of Rajasthan and its energy potentials. J. Enviroment and pollution. 2(3), 105-109.

Sanghi, S. and A. Kumar (2000): Characterization of some of the medicinal plants of family Leguminosae used in Ayurvedic system of medicines. Int. J. Mendel. 17, 109-110.

Sharma, L.K. and A. Kumar (2000): Searching for anti-cancer drugs in traditional medicines. Int. J. Mendel. 17, 77-78.

Sharma, S. and A. Kumar (2001): Ayurvedic plants for cure of hepatic diseases. Int. J. Mendel. 18: 13-14.

Sharma, V. and A. Kumar (2001): Studies on anti-paralytic crude drugs used in Ayurvedic system. Int. J. Mendel. 18, 33-34.

Shivani and A. Kumar (2000): Ayurvedic medicinal plants used for skin diseases. Int. J. Mendel. 17, 105-106.

Wan , M., S. Bloor, L. Foo and B. Loh (1996): Screening of New Zealand plant extracts for inhibitory activity against HIV-1 protease. Phytother. Res. 10, 589-595.

Yadav, A. and A. Kumar (2001): Studies on anti-malarial drugs used in Ayurvedic system. Int J. Mendel Int. J. Mendel. 18(1-2), 29-30.