African Journal of Biotechnology Vol. 8 (23), pp. 6569-6575, 1 December, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Determination of chilling temperature effects on nutrient elements composition and distribution in cole (*Brassica oleracea* L. Cv. Acephala) using the WDXRF spectroscopic technique

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Accepted 13 August, 2009

Cole (*Brassica oleracea* L. cv. Acephala) is a naturally very hardy species to (at) chilling temperatures. It has been observed that the plant species can be viable even under snow during a cold winter. The cole seedlings were grown in soil for one month. Chilling temperatures were then applied to these seedlings under controlled conditions. These seedlings were subsequently, cut into root tip, root middle part, root upper part, hypocotyl, epicotyl, petiole and leaf and sampled randomly. Concentrations of inorganic elements (Al, Si, P, S, Cl, K, Ca, Fe, and Mg) in the parts were measured by wavelength-dispersive X-ray fluorescence (WDXRF) spectrometry to test chilling temperature effects on nutrient accumulation and distribution within these seedlings. Results indicated that the distribution of some inorganic elements among organs (roots, stem and leaves) of cole plants is significantly altered by chilling stress. There was an association between chilling temperatures and distributions, and accumulations of Ca, Fe, P, Cl, S especially Si and Al in cole seedlings. In addition, the WDXRF technique is a simple, fast, economic and accurate tool for biological studies related to the determining of the amount of plant nutritions in ppm level.

Key words: Common cole (*Brassica oleracea* cv. Acephala), inorganic element, chilling temperature, X-ray, WDXRF.

INTRODUCTION

Cole is one of the most commonly consumed vegetables by people living in the Trabzon Region of Turkey due to its ability to grow all year round (Tirasoglu et al., 2005). Chilling stress, or the damage caused by low but above-freezing temperatures, has been recognized as a critical environmental factor affecting crop growth. Chilling stress may significantly influence plant growth, resulting in considerably yield reductions in the cultivation areas at higher latitudes (Laura et al., 2004).

There are very close relations between inorganic elements and the metabolism of living organisms. For example, calcium (Ca) is required for the normal functioning of plant membranes. In addition, it acts as a messenger to regulate plant growth and development, as well as adaptation to environmental stresses (Poovaiah and Reddy, 1993; Bush, 1995). Silicon (Si) can stimulate growth and yield by decreasing mutual shading, causing leaf erectness, decreasing susceptibility to lodging, decreasing the incidence of fungal infections, and preventing Mn and/or Fe toxicity in plants (Salvador et al., 2002).

The recent advent of commercially available wavelength-dispersive spectrometers for X-ray fluorescence (WDXRF) measurements has provided an economical and powerful tool for environmental, clinical, chemical, geological and industrial analysis. WDXRF is a non-destructive, fast, multi-element technique for analyz-

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ing the surface layers and determining major, as well as minor elements, in thin and thick samples of all sizes and forms. Demir et al. (2008) investigated the accuracy of WDXRF spectroscopy used in their study.

A wide range of new research applications has been demonstrated by using the energy dispersive X-Ray fluorescence (EDXRF) technique (Cevik et al., 2003; Perring et al., 2005; Karabulut et al., 2005; Noda et al., 2006; Fu et al., 2006; Cabrera et al., 1996), similar to wavelength dispersive X-Ray fluorescence (WDXRF) technique, for measuring element contents in different organic materials. In both techniques, X-ray principles are used. X-ray spectrometry techniques have been used for determination of macro-microelement contents in biologic materials (e.g., in some commercial food materials (Perring et al., 2004; Alvarez and Mazo-Gray, 2005) in some plants, (Tirasoglu et al., 2005; Demir et al., 2006; Noda et al., 2006; Fu et al., 2006; Perez et al., 1991) and in insects (Dumlupinar et al., 2006; Erman et al., 2006). Fu et al. (2006) demonstrated that Ca levels were different in various tissues and organs of *Chorispora bungeana* and they suggested that these differences of Ca were connected with cold-hardiness mechanism of the plant species by using EDXRF. In our previous study, we reported that the distribution of inorganic elements among organs of a susceptible plant (*Phaseolus vulgaris* L.) is significantly altered by chilling stress and we noticed that the WDXRF technique is reliable for measuring inorganic elements content (contained or present in or the inorganic elements content of) of plant tissues in ppm level (Dumlupinar et al., 2007).

Our previous findings showed that K, Cl, P, S and especially Cu are significantly exchanged between pulvinus and neighbor tissues during nyctinasty movement due to chilling stress in bean plants (Dumlupinar et al., 2007). We hypothesized that these elements could be responsible for the occurrence of nyctinasty movement in bean plants. We supposed that the results of the researches will be able to supply some clues on the determining of tight relations between especially some inorganic elements whose roles are previously unknown and metabolic pathways activated at chilling temperatures in plants. This is the first report on the chilling resistance of *Brassica olerace* L. cv. Acephala (cole) that attempts to determine the distribution and accumulation of inorganic nutrient elements in each vegetative organ at chilling temperatures. The objectives of this study were to examine the effect of chilling stress on the accumulation and distributions of nutrient elements within the cole plant using the WDXRF technique.

MATERIALS AND METHODS

Instrumentations

A wavelength-dispersive spectrometer (WDXRF, Rigaku ZSX-100e with Rhodium target X-ray) was used. This instrument was controlled by a Software ZSX computer.

Plant material

Cole seedlings (*B. oleracea* L. cv. Acephale) were used in this study. Seedlings were grown in 3-L glazed pots filled with soil and with approximately 10 seedlings per pot. Initially, seeds were sprouted at 20°C in the dark. Seedlings were grown at 25°C in the light: 20°C in the dark under a 16 h photoperiod controlled-environment for 1 month. Acclimation was achieved at 15°C in the light: 10°C in the dark under a 14 h photoperiod for 1 day. Then, these seedlings were exposed to 5°C in the light: 2°C in the dark under a 12 h photoperiod for 9 days. Seedlings, which had not been treated with acclimation and cold, were used as a control.

Sample preparation

After treating with chilling temperature, these plant tissues were washed three times in triple distilled water in the laboratory. After drying at 60°C to a constant weight, the dry tissue material was ground by hand in a ceramic mortar with liquid nitrogen to make the samples as homogenous as possible. Each sample was 2 g. The samples were then transferred into a 3.0 cm diameter disc of polyethylene mylar film. To obtain a XRF-mylar film, a small metallic sample holder made of aluminum with a diameter of 3.0 cm was used.

Measurements

SQX advanced semi-quantitative software of wavelength-dispersive X-ray fluorescence spectrometry was used for analysis. Concentrations of Carbon and other basic elements (H, O, N) were not considered in our measurements. Results of measurements which were taken by WDXRF were compared to each other as a part of percent (%) in this study. The measurements were performed using a ZSX 100e sequential spectrometer equipped with a Rh X-ray tube. The apparatus working conditions are shown in Table 1.

RESULTS AND DISCUSSION

The WDXRF technique was used to determine the level of nutrient elements in organs of the cole (*B. oleracea* L. cv. Acephala) seedlings in this study. It has been reported that this technique does not need to use any acidic chemical substance to measure the content of the organic/inorganic elements in the biological samples and it takes lesser time than the chemical methods in previous studies (Karabulut et al., 2005; Dumlupinar et al., 2007; Dumlupinar et al., 2008). Samples were prepared by using only mechanical procedures (see plant material part). Original figures of normalized concentration versus parts of both control and chilling biological samples of Al, Si and Ca elements can be seen in our measurements using WDXRF (Figure 1a, b, c).

Concentrations of Si were relatively higher in the root part of the cole control seedlings when compared to chilled seedlings (Table 2a). Whereas, Si concentrations were lower in the leaves, especially in petioles and epicotyls. The concentration of Si showed an increment in the leaves (65%), by the time it generally decreased in the under part of the plants (especially in petioles, hypocotyls, epicotyls and root upper parts; 99, 69, 15 and

Table 1. Experimental conditions for the analysis of eight chemical elements.

Element	Line	Crystal	Attenuator	Slit	Detector	V (kV)	I (mA)
Ca	Kα	LiF	1—1	STD.	Flow	40	90
Si	Kα	PET	1—1	STD.	Flow	30	90
S	Kα	GE	1—1	STD.	Flow	30	90
P	Kα	GE	1—1	STD.	Flow	30	90
Cl	Kα	GE	1—1	STD.	Flow	30	90
K	Kα	LiF	1—1	STD.	Flow	40	90
Fe	Kα	LiF	1—1	STD.	Scintillate	50	72
Mg	Kα	LiF	1—1	STD.	Scintillate	50	72

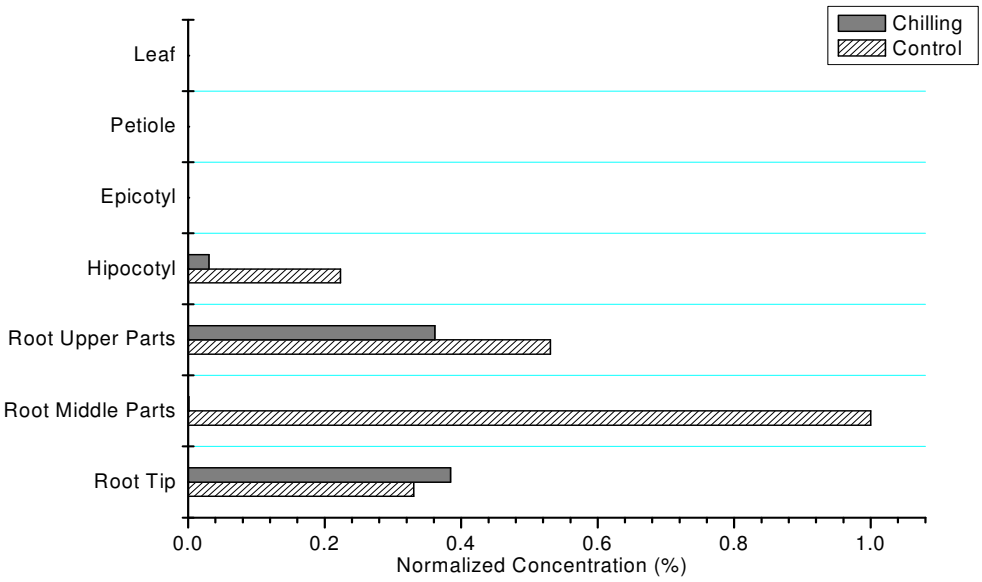

Figure 1a. Normalized concentration versus parts of both control and chilling biological samples of Al elements.

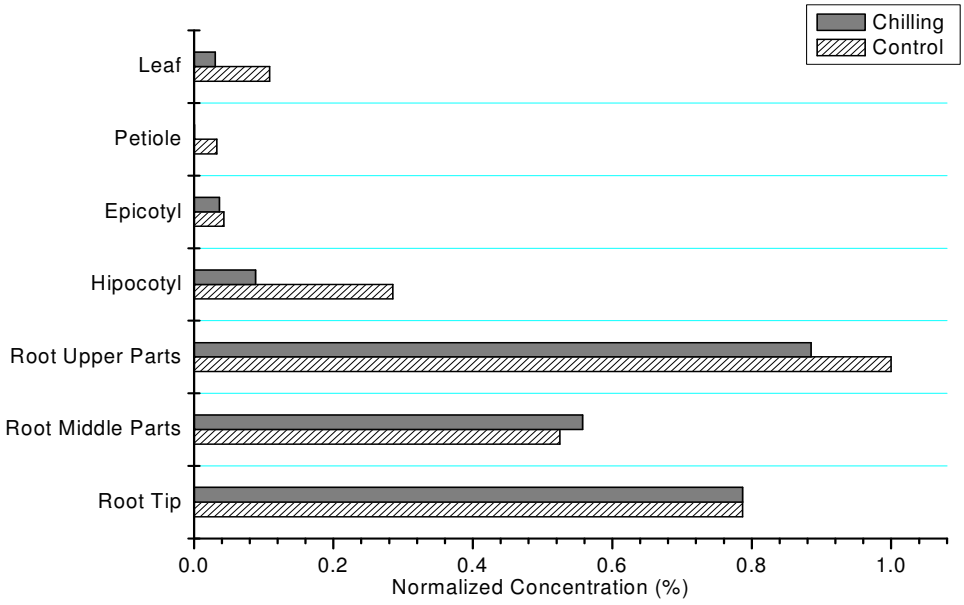

Figure 1b. Normalized concentration versus parts of both control and chilling biological samples of Si elements.

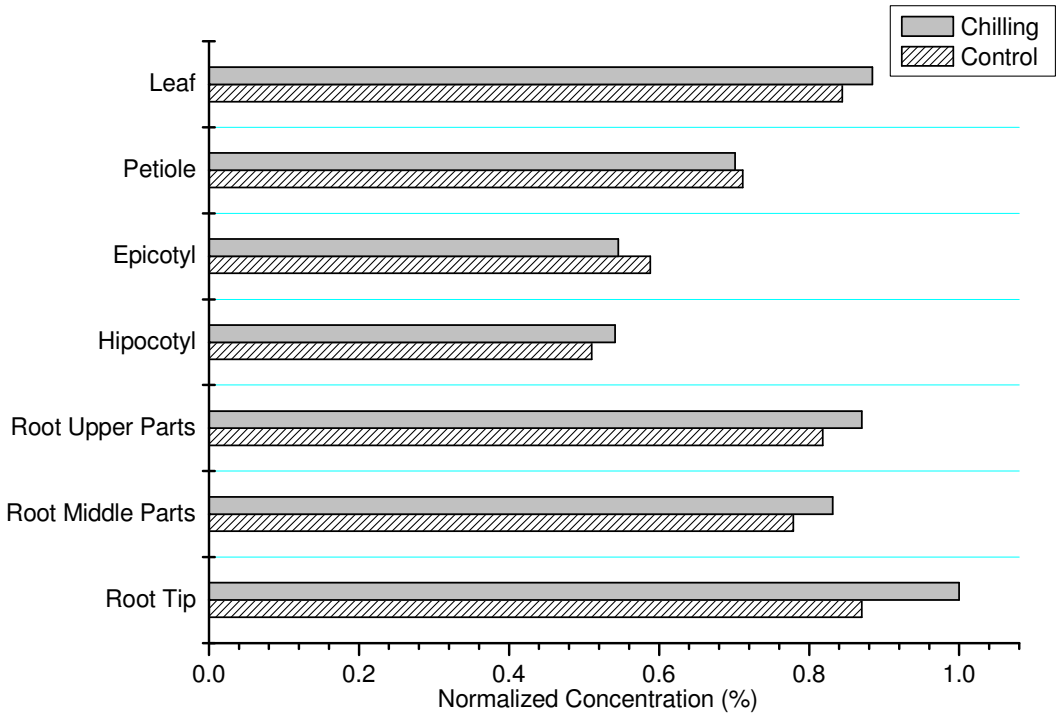

Figure 1c. Normalized concentration versus parts of both control and chilling biological samples of Ca elements.

Table 2a. Concentrations of inorganic elements obtained from root parts of cole.

Element	Concentrations % (ppm)								
	Root tip			Root middle parts			Root upper parts		
	Control	Chilling	Change (%)	Control	Chilling	Change (%)	Control	Chilling	Change (%)
Al	0.430	0.500	14.0	1.300	0.001	-99.9	0.690	0.470	32.0
Si	2.400	2.400	0.0	1.600	1.700	6.0	3.050	2.700	11.0
P	1.500	0.950	36.6	6.200	2.400	61.0	1.090	1.000	8.0
S	4.200	2.800	33.0	6.200	5.300	15.0	4.260	3.700	13.0
Cl	0.001	1.800	99.9	----	----	----	1.230	1.400	12.0
K	16.000	8.700	45.6	21.000	16.000	29.0	19.100	17.000	-11.000
Ca	67.0000	77.000	13.0	60.000	64.000	6.0	63.000	67.000	6.0
Fe	8.100	6.100	25.0	10.000	11.000	9.0	7.570	7.200	5.0

11%, respectively). Namely, Si translocated from the under parts to the leaves of cole seedlings and Si was accumulated in both leaves and tips of the roots under chilling stress. Whereas, we determined that Si moves from the leaves and the other upper parts of beans to the roots and accumulated in the roots in the same environmental conditions in our previous work (Dumlupinar et al., 2007). There is lack of knowledge about the effect of Si on plant chilling stress metabolism, but it is known that silicon affects vegetative growth to a much lesser extent than it does to a reproductive growth in plants. It has been reported that the stimulation of growth by Si can also be caused by the prevention or depression of manganese and iron toxicity. In addition, Si increases leaf erectness (Salvador et al., 2005). We observed that leaves of cole seedlings could protect their erectness during chilling stress, in contrast to findings which we obtained from bean seedlings in our previous work. These findings suggest the possibility of a relationship between tissue Si content and the cold hardiness of cole plants.

Sulfur concentrations decreased in all root parts and hypocotyls, whereas they statistically did not change in epicotyls and leaves. We determined that the total S level was lower in the chilled whole cole plants in the control (Table 3). These findings led us to propose that some nutrient elements may exhibit altered distribution among

Table 2b. Concentrations of inorganic elements obtained from stem parts of cole.

Element	Concentrations % (ppm)					
	Hipocotyl			Epicotyl		
	Control	Chilling	Change (%)	Control	Chilling	Change (%)
Al	0.290	0.040	86.0	----	----	----
Si	0.870	0.270	69.0	0.130	0.110	15.0
P	0.970	0.750	23.0	0.750	0.810	7.0
S	3.800	3.340	12.0	5.290	5.100	4.0
Cl	5.800	5.090	12.0	7.190	5.700	20.0
K	45.000	47.800	6.0	41.300	46.000	10.0
Ca	39.300	41.700	6.0	45.300	42.000	7.0
Fe	3.930	0.970	75.0	----	----	----
Mg	----	----	----	0.001	0.750	99.9

Table 2c. Concentrations of inorganic elements obtained from leaf parts of cole.

Element	Concentrations % (ppm)					
	Petiole			Leaf		
	Control	Chilling	Change (%)	Control	Chilling	Change (%)
Al	----	----	----	----	-----	----
Si	0.100	0.001	99.0	0.330	0.940	65.0
P	0.540	0.610	11.0	0.780	0.950	18.0
S	3.470	3.500	1.0	6.300	6.010	5.0
Cl	9.210	6.800	26.0	8.600	7.270	15.0
K	31.900	35.000	9.0	19.000	16.700	12.0
Ca	54.800	54.000	1.0	65.000	68.100	5.0
Fe	----	----	----	----	----	----
Mg	----	----	----	----	----	----

*Means in the same line for every part of plant, followed by the same letter are not significantly different at the ($P < 0.05$) level.
Concentrations of carbon and other basic elements (H, O, N) were not considered in our measurements in this study. Results of measurements which were taken by WDXRF were compared to each other as a part of percent (%).

Table 3. Change of total concentrations (%) of inorganic elements in whole cole plants.

Parameter	Al	Si	P	S	Cl	K	Ca	Fe	Mg
Control	2.710	8.150	11.830	33.520	32.030	193.300	394.400	29.600	0.001
Chilling	3.720	8.120	19.540	29.750	28.060	187.200	413.800	25.300	0.750
Change (%)	27.000	-0.400	39.000	-11.000	-12.000	-3.000	5.000	-15.000	99.900

Concentrations of carbon and other basic elements (H, O, N) were not considered in our measurements in this study. Results of measurements which were taken by WDXRF were compared to each other as a part of percent (%).

organs, but also they may be exchanged between roots and soil as a reaction because of chilling stress. But we cannot say whether the element distributions are occurring to supply chilling resistance by cole plant in this current stage or not.

Phosphorus concentrations showed a significant decrease in root tips and hypocotyls, while they increased especially in epicotyls and leaves (Table 3). These findings are completely in contrast to results which we had for P in bean plant in our previous work (Dumlupinar et al., 2007).

The Chloride content could not be determined only in middle parts of roots of the control and chilled plants. It was determined that Cl moved from the upper organs to the roots (especially to the tip of the roots) (Table 3). We realized that an amount of Cl is discharged to outside of

the plant via root because we found that its total change in percent (%) was lower in the chilled whole plants than the control plants. In our previous work, it was determined that Cl moved from the epicotyls to the leaves of beans (Dumlupinar et al., 2007).

Potassium concentration decreased in root parts and leaves, whereas it showed accumulation in hypocotyls, epicotyls and petioles, but total percent concentration of K statistically did not change in the whole cole plants (Table 3). This means that K was just distributed between organs, but it was not charged or discharged in this plant. On the other hand, when the K and Ca concentrations were considered, we determined that Ca was the highest concentration both totally (193:394, K:Ca total (%), respectively) (Table 3) and in all parts of organs (except for hypocotyl; 45:39, K:Ca %) in control plants and chilling plants (except for hypocotyl 48:42, K:Ca %, respectively and epicotyl; 46:42, K:Ca%, respectively) (Table 2b). Tirasoglu et al. (2005) reported that K concentration was the highest concentration in cole under field conditions by using the EDXRF technique.

We determined that calcium concentration increased in hypocotyls, leaves and in the root parts (especially in the tip of the root, 13%) (Table 2a). In our previous work, we had found that Ca had increased especially in epicotyls, petiole and leaves, but Ca concentration had decreased in all root parts in beans (Dumlupinar et al., 2007). Therefore Ca concentration increased in this cold resistant plant (cole) in the roots and it decreased in susceptible plants (for example, beans) in comparison with control plants under chilling temperatures. In addition, Fu et al. (2005) investigated the content of Ca, P, K, Mg, S and Cl on *C. bungeana* that is capable of resisting a freezing environment after chilling temperatures. They reported that Ca accumulation and distribution changes were involved in the cold hardiness of leaves, petioles and roots. They demonstrated that the difference in Ca distribution was the response of *C. bungeana* in adapting itself to the alpine subnival environment and the accumulation of Ca could play an important role in its active cold-hardiness. Our result was in agreement with Fu et al. (2005)'s findings. Further, we think some other inorganic elements also play a role in the cold hardiness metabolism of plants.

The iron content could be determined only in root parts and hypocotyls (Table 2a, b). Its concentration showed increment only in the middle parts of the roots, while its concentration decreased in the other parts (the tip and upper part of the root and hypocotyls) of the cole plants in comparison with the control plants. By the way, when any value of an element can not be found in a sample using an X-ray technique, it can not be assumed that there is certainly (definitely) no element in that sample. These X-ray systems can relatively measure the concentration of each element in a sample. Therefore if some element concentrations are high, the systems can not sensitively measure other elements with very low concentrations. On the other hand, it is well known that Fe is one of the essential inorganic elements in plants. It has very important roles in living metabolism. We can say that there are low concentrations of Fe (and some other inorganic elements) which we did not mention in the all parts of the cole plant, too. But they are very small amounts as much as which we can not even measure using the X-ray techniques which are known to be the most sensitive ones in the present time. So, what is the meaning of decreasing of Fe concentration in the organs of the cole plant? We suggest that reducing the Fe content in the plant organs can cause decreasing in rates of respiration and photosynthesis. Thus, we may assume that respiration is getting at a low level in the root of the cole seedlings under chilling temperatures.

The aluminum content could only be measured in root parts and hypocotyls of the cole plants (Table 2a, b, c). Its concentrations were increased in only the root tips and especially in the hypocotyls in comparison with the control plants.

It has been recorded that concentrations of many inorganic elements changed in different organs under chilling stress conditions in plants (Fu at al., 2006; Dumlupinar et al., 2007). We also hypothesized here that concentrations of many nutrient elements changed between organ parts under chilling stress conditions in the cole plant, too. We suggested here that the replacements and accumulations of measured elements are different in cold resistant plants, like the cole in comparison with non-cold resistant species, like bean plants. The differences were determined in especially Ca, Fe, P, Si, Al, Cl and P contents (Table 2a, b, c). We estimate that the contrast directions of these element movements can be connected with the resistance of the cole plant. We think that the plant can specifically regulate the distribution and accumulation of inorganic elements in its own body at chilling temperatures and thus, they can up-regulate their metabolic activities by using the elements against chilling stress. Our findings suggest here that cole has some appropriate genetic mechanisms supplying distribution and accumulation of these elements against chilling stress.

Conclusion

The distribution and accumulation of measured nutrient elements among the organs of cole plants were significantly altered by chilling stress. It is necessary to carry out research on more plant species in both cold resistant and sensitive species to support the results. Future research is needed on more plant species to understand the biochemical mechanisms involved in cold resistance. Determination of the distribution of inorganic elements among plants and theirs outside may supply some clues that show which metabolic pathways become active or passive under chilling stress conditions. In addition, determining of decreasing or increment of the inorganic

element concentrations in a chilling resistant plant, like cole, may show us which element's increment or decreasing is needed for chilling resistance mechanism in the plants. We believe that obtaining this knowledge may help us change usual plant fertilization regimes resistance of agricultural plants which are sensitive to chilling temperatures in field. By the way, using the WDXRF technique as a simple, fast and accurate tool can significantly improve the efficiency of nutrient studies in plant physiology and enhance our knowledge of plant physiology in regard to cold resistance mechanisms in plants.

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