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Physiological and Biochemical Mechanisms of Plant Adaptation to Low-Fertility Acid Soils of the Tropics: The Case of Brachiariagrasses

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1. Introduction

Brachiaria species are the most widely planted tropical forage grasses in the world (Miles et al., 2004). For example, in Brazil alone, about 80 million hectares are planted to *Brachiaria* pastures (Macedo, 2005). They increase animal productivity by 5 to 10 times with respect to native savanna vegetation in the tropical areas of Latin America, thus representing a significant contribution to farmer's income (Rao et al., 1993). Although their origin is from the tropical areas of Africa, they are also used for livestock production in South-East Asia and Australia. Among them, *Brachiaria decumbens* cv. Basilisk, *Brachiaria brizantha* cv. Marandu, and *Brachiaria ruziziensis* cv. Kennedy have been more commonly utilized for livestock production in the tropics (Miles et al., 2004). Among the three grasses, *B. decumbens* is highly adapted to infertile acid soils, i. e., high level of tolerance to high aluminium (Al) saturation, low phosphorus (P) and low calcium (Ca) supply in soil (Louw-Gaume et al., 2010 a,b; Rao et al., 1995, 1996; Wenzl et al., 2001, 2003), but also highly sensitive to a major insect, spittlebugs (Miles et al., 2006) and produces mycotoxin after infection with *Pithomyces chartarum* (Andrade et al., 1978). *B. brizantha* cv. Marandu is highly resistant to spittlebugs, adapted to seasonal drought stress, highly responsive to fertilizer application but is not well adapted to low fertility acid soils (Miles et al., 2004, 2006). *B. ruziziensis* cv. Kennedy is sensitive to spittlebugs, performs better in well-drained fertile soils, has high forage quality but poorly adapted to low fertility acid soils (Ishigaki, 2010; Miles et al., 2004). *B. decumbens* and *B. brizantha* are generally tetraploid, apomicts while *B. ruziziensis* is diploid, sexual (Miles et al., 2004, 2006).

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CIAT and its collaborators have an on-going breeding program to combine the desirable attributes from the three grasses (Miles et al, 2004, 2006). *B. hybrid* cv. Mulato is the product of three generations of crosses between *B. ruziziensis*, *B. decumbens* and *B. brizantha*. This grow well in low P, low fertility acid soils in both wet and dry seasons (Rao et al., 1998), and produces a large numbers of panicles with well synchronized flowering and good caryopsis formation, which leads to good-quality seed. Defining the specific physiological and biochemical mechanisms that are associated with greater adaptation to low fertility acid soils will contribute to developing rapid and reliable methods to select the phenotypes and to develop molecular markers for marker assisted breeding of brachiariagrasses. Developing superior *Brachiaria* hybrids from the on-going breeding programs that combine the desirable attributes including adaptation to major biotic and abiotic constraints, forage quality, and seed production will facilitate sustainable intensification of crop-livestock systems in the tropics (Miles et al., 2004, 2006; Rao, 2001 a,b). This chapter reviews the progress made in defining the physiological and biochemical mechanisms of adaptation of brachiariagrasses to low fertility acid soils.

There is limited knowledge on the comparative differences in Al resistance among *B. decumbens*, *B. brizantha*, *B. ruziziensis* and *B. hybrids* (Mulato and Mulato II) grown in hydroponic system. Identification of plant attributes that contribute to greater ability to acquire nutrients under low pH, low P and high Al conditions is critical to develop brachiariagrasses that are productive and persistent under infertile acid soil conditions.

There have been some discussions on the validity of short-term screening technique that uses simple solution of Al and Ca to test the effect of Al on relative root elongation of young seedlings: whether the results obtained by this short-term screening technique can apply to the behaviour of older plants is under discussion (Ryan et al., 2011). However, significant positive correlations were observed on Al resistance of 15 cultivars of sorghum (*Sorghum bicolor* Moench) and 10 cultivars of maize (*Zea mays* L.) with data obtained using short-term (1 day) screening and long-term screening technique using hydroponic system (Akhter et al., 2009). Similar results were also observed with 8 rice cultivars (unpublished data). In this chapter, we consider short-term vs long-term responses of several plant species including brachiariagrasses that differed widely in their level of Al resistance.

B. decumbens is known for very high level of Al resistance, however, the mechanisms responsible for this high level of Al resistance was not associated with exudation capacity of organic acid anions from root tips (Wenzl et al., 2001). It is important to define the specific mechanism(s) contributing to the high level of Al resistance in *B. decumbens*.

Higher level of Al exclusion was found in *B. decumbens*, however, the specific mechanisms related to Al exclusion are not known. Several mechanisms other than organic acid anion exudation were found and the related Al resistance genes have been reported (Huang et al., 2009; Yamaji et al., 2009; J.L. Yang et al., 2008; Z-B Yang et al., 2010). We found a new mechanism for higher level of Al tolerance of an Al-resistant rice cultivar based on higher abundance of sterols in plasma membrane (PM) lipids of root-tip cells (Khan et al., 2009). Rice is also known for its greater level of Al resistance than other cereal crops and its level of resistance was also not related to organic acid anion exudation (Ishikawa et al., 2000; Ma et al., 2002). Therefore, it is crucial to test the role of sterols in PM lipids of root-tip cells of brachiariagrasses.

High concentrations of phenolic compounds have been reported as one of the promising mechanisms to explain higher level of Al resistance of a forage legume, *Lotus pedunculatus* Cav. (Stoutjesdijk et al., 2001) and several common woody plants (Ofei-Manu et al., 2001).

This can be explained by higher complexing abilities of phenolic compounds with Al ions (Cornard & Merlin, 2002; Yoneda & Nakatsubo, 1998). Phenolic compounds have also been reported to be solubilized into lipid layer (Boija et al., 2006, 2007). Existence of phenolic compounds in lipid layer was found to make PM less fluid (Arora et al., 2000). Lipid layer with less fluidity will make PM less permeable even in the presence of Al ions (Khan et al., 2009). The contribution of phenolic compounds in root-tip portion to high level of Al resistance in *B. decumbens* is not known.

A major constraint to agriculture on tropical and subtropical soils is P deficiency (Fairhurst et al., 1999). Applying large amounts of fertilizer to correct P deficiency is not feasible for most resource-poor farmers in developing countries. Thus the agricultural productivity becomes limited in the near future. Moreover, P fertilizer is receiving more attention as a nonrenewable resource (Cordell et al., 2009; Steen, 1998). For sustainable P management in agriculture on tropical and subtropical soils, it is essential to define the mechanisms involved in making plants more efficient in P acquisition and use (Lynch, 2011; Ramaekers et al., 2010; Rao et al., 1999).

P is a constituent of phospholipids (PL), nucleic acids, nucleosides, coenzymes, and phosphate esters in plants. P helps regulate plant metabolisms by controlling enzymatic activity through phosphorylation and/or dephosphorylation. To overcome P deficiency, plants develop several strategies, including the well-known one of secreting acid phosphatase (APase), ribonuclease (RNase), and organic acids into the rhizosphere to improve P availability in the soil (Duff et al., 1994; Green, 1994; Jones, 1998; Rao et al., 1999; Tadano et al., 1993; Wasaki et al., 2003a). Besides from acquiring P from the outside of the plant, APase is also recognized for its role in efficient utilization of absorbed P for metabolism (Duff et al., 1989, 1991). One of the mechanisms to increase P recycling ability is dependent on the activity of APase (Duff et al., 1991, 1994) and RNase (Howard et al., 1998) in the cell. The increase of both enzymes is reported and these enzymes are considered to utilize those P compounds that are stored in vacuole. The other mechanism is bypassing several metabolic pathways to reduce the usage of P molecule. Theodorou and Plaxton (1993) showed that P deficiency induces some glycolytic enzymes, such as phosphoenolpyruvate carboxylase (PEPC) and phosphoenolpyruvate phosphatase (PEPP). These catalyze the bypass reaction of pyruvate kinase (PK), which is responsible for regulating carbon flow from glycolysis to the TCA cycle. PEPC replenishes intermediates of the TCA cycle, and may help regulate both carbon and nitrogen metabolism, and P recycling under P deficiency. Kondracka and Rychter (1997) observed that, in P-deficient bean leaves, the rate of malate synthesis increases, and the accumulation of aspartate and alanine (products of PEP metabolism) is also enhanced. In early stages of P deficiency, the increased activity of PEPC and use of PEP in amino acid synthesis are probably the most important reactions for P recycling in bean leaves during photosynthesis. Thus, PEP metabolism by PEPC and PEPP, or PEP transport via PPT (which transports PEP from the cytosol into chloroplasts in leaves) may affect carbon distribution within the plant under P deficiency.

Under P-deficient conditions, the brachiariagrasses improve their P acquisition by enhancing root growth, uptake efficiency, and ability to use poorly available plant P (Louw-Gaume et al., 2010b; Rao et al., 1999, 2001a). Although they have much lower internal requirements for P than do other grasses, they also show interspecific differences (Rao et al., 1996).

Recent advances in post-genomic studies have indicated that transcriptomic analysis is a useful tool for understanding gene expression networks. Some transcriptomic studies of

low-P adaptation strategies have also been carried out using cDNA arrays (Hammond et al., 2003; Misson et al., 2005; Ramaekers et al., 2010; Uhde-Stone et al., 2003; Wang et al., 2002; Wasaki et al., 2003b, 2006; Wu et al., 2003). Although some aspects of plant strategies for coping with P-deficient conditions are understood, the majority are not; a broad view of gene expression is necessary to fully elucidate all of the mechanisms involved (Ramaekers et al., 2010). In this chapter, we review the progress in understanding the transcriptomic changes by P deficiency in rice plants, which is a model of Gramineae plants and also relatively tolerant of low P and low pH conditions. We use qRT-PCR for quantification of the effects of P deficiency on phosphohydrolases and carbon metabolism in rice leaves. We also review the progress in defining the physiological and biochemical bases of improved P use efficiency in *B. hybrid* (cv. Mulato).

2. Materials and methods

We randomly selected seven plant species, i.e., rice (*Oryza sativa* L. cv. Sasanishiki), maize (*Zea mays* L. cv. Pioneer 3352), pea (*Pisum sativum* L. cv. Kinusaya), barley (*Hordeum vulgare* L. cv. Manriki), tea (*Camellia sinensis* L. cv. Yabukita), siclepod (*Cassia tora* L.) and *B. brizantha*. Seeds of rice, maize, pea were soaked in tap water under aeration for 3 to 24 h depending on plant species. Seeds of siclepod were notched by a razor to facilitate germination and soaked in tap water for 12 h. Seeds of barley and *B. brizantha* were not soaked. These seeds were germinated on a nylon screen that was put on a polypropylene container filled with tap water under aeration at 27°C in a growth room. Seeds of tea were germinated in quarts sand wetted with deionized water, and then seedlings with roots approximately 1 cm long were transferred to the container described above. All the seedlings with roots approximately 5 cm long were used in the following experiments. In the short-term experiment, ten seedlings of each plant species were pretreated in 600 mL volume of a solution with (Al treatment) or without (control) 50 μ M AlCl_3 containing 0.2 mM CaCl_2 at pH 4.7 for 1 h. After measurement of the root length with a ruler, all the seedlings were transferred into a Al-free 0.2 mM CaCl_2 solution at pH 4.7. Root length was measured again after 24 h. For the tea plant, the root length was measured after 3 d because of the slower root elongation than in the others. Relative growth (%) in the short-term treatment with Al was calculated as the ratio of net root re-elongation of the primary root in the Al treatment to that in the control. In the long-term experiment, twenty seedlings of each plant species were precultured in a 54-L volume of nutrient solution at pH 5.2. The nutrient solution was composed of 1.43 mM NH_4NO_3 , 0.7 mM NaNO_3 , 0.13 mM NaH_2PO_4 , 0.78 mM K_2SO_4 , 1 mM CaCl_2 , 0.6 mM MgSO_4 , 36 μ M FeSO_4 , 9 μ M MnSO_4 , 0.08 μ M CuSO_4 , 0.03 μ M $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, 18.5 μ M H_3BO_3 , 1.5 μ M ZnCl_2 . After 1 week, the seedlings were transferred into the nutrient solution with same composition as above (control) or the nutrient solution containing 100 μ M Al and 10 μ M P in soluble form (Al treatment) at pH 5.2 or 4.5, respectively. The solutions were renewed weekly. The concentrations of Al and P, and pH of the solution were monitored every day and adjusted if required. Two months after the Al treatment, the plants were harvested and dried in a draft oven (60°C). Relative growth (%) in the long-term treatment with Al was calculated as the ratio of the dry weight of whole plant in the Al treatment to that in the control. The concentrations of K, P, Mg, and Ca were determined by ICP-AES (inductively coupled plasma-atomic emission spectrometry) after digestion of the plant samples using an acid mixture (HNO_3 : HClO_4 = 5: 3, v/v). Extraction and determination of the phenolic compounds were carried out as described by Ofey-Manu

et al. (2001). Seedlings of *Brachiaria* hybrid (*B. ruziziensis* Ger. & Ev. clone 44-06 × *B. brizantha* (A. Rich.) Stapf CIAT 36061, also known as cv. Mulato), *Andropogon gayanus* Kunth (CIAT 621), barley (cv. Ryofu) were transferred to 36-L containers containing nutrient solution with or without 0.37 mM Al (as $\text{Al}_2[\text{SO}_4]_3$). At the end of 10 days of treatment, the roots of seedlings from each treatment were sampled, dried in a forced-air oven at 80°C for 72 h, and then weighed. The dried samples were ground and digested with $\text{H}_2\text{SO}_4\text{-H}_2\text{O}_2$ for Al analysis by ICP-AES. *Brachiaria* seedlings were prepared as described above, and transferred to 36-L containers carrying the standard nutrient solution, but with 2.8 mM Al at pH 3.7 added, and left to grow for 1 month. The much higher Al concentration was used to ensure clear peaks in the ^{27}Al NMR spectrum. Even so, the *Brachiaria* seedlings grew well (data not shown). After treatment, roots were removed from the seedlings and washed, first with tap water, then with deionized water. The roots were grouped into three: Fraction (a), roots given the water washings only, and used to determine total amounts of Al and organic acid anions; and Fraction (b), roots were also washed with 0.1 M HCl for 5 min to remove apoplastic, soluble or loosely bound, components. Each fraction of *Brachiaria* roots was placed in a 10-mm-diameter NMR tube. AlCl_3 (0.1 M) solution was used as an external reference to calibrate the chemical shift (0 ppm). ^{27}Al NMR spectra were recorded, using a Bruker MSL400 spectrometer at 104.262 MHz. The spectra were obtained by using a frequency range of 62.5 kHz, a pulse width of 12 μs , a delay time of 0.16 ms, a cycle time of 0.5 s, and 4000 scans. For estimation of low P tolerance, we have selected *Brachiaria* hybrid (cv. Mulato) and rice (*Oryza sativa* L. cv. Nipponbare). For the seedling growth nutrient solution was prepared with 2.12 mM N (NH_4NO_3), 0.77 mM K ($\text{K}_2\text{SO}_4\text{:KCl} = 1\text{:}1$), 1.25 mM Ca ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), 0.82 mM Mg ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 35.8 μM Fe ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), 9.1 μM Mn ($\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$), 46.3 μM B (H_3BO_3), 3.1 μM Zn ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), 0.16 μM Cu ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), 0.05 μM Mo ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$), with 6 μM P ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$) and pH was maintained at 5.2 for 1 week preculture. Then phosphorus level was changed as 0, 6 and 32 μM for 2 weeks. After sampling, samples were freeze-dried, then P fractionation was carried out based on Schmidt-Thannhauer-Schneider method. Samples were also used for measurements of APase and RNase activities. $^{14}\text{CO}_2$ was generated by adding 30% PCA into $\text{NaH}^{14}\text{CO}_2$ (18.5 kBq), then applied to plants for 5 min in a vinyl package under natural light condition. After sampling, samples were fractionized by using column method to obtain organic acids, amino acids, and sugars fraction, then ^{14}C content in each sample was determined by using scintillation counter. In transcriptomic analyzes and subsequent molecular analysis, we have selected rice (*Oryza sativa* L. cv. Michikogane) and the growth condition was same as shown before except for the P treatment was done using 0 or 32 μM levels. Total RNA was extracted from frozen samples using a sodium dodecyl sulfate (SDS)-phenol method. The real-time PCR was performed by the LightCycler™ system (Roche) with the LightCycler DNA Master SYBR Green I kit for PCR (Roche), and the TaqStart™ antibody (Clontech) was used for the repression of unspecific amplification.

2.1 Mechanisms of Al resistance in brachiariagrasses

2.1.1 Differences in Al resistance and nutrient acquisition among crops and brachiariagrasses

Differences in Al resistance among crops and *B. brizantha* are shown in Fig.1 (the left panel). Al resistance was ranked as follows: *B. brizantha* > rice > tea > maize > pea, sicklepod > barley. The order of Al resistance in the short-term experiment was well correlated with that of the

long-term experiment in spite of marked difference in the treatment conditions, i.e., duration of Al treatment, Al concentration, composition of co-existing nutrients, pH, etc. ($R^2 = 0.785$ [$p < 0.01$], right side figure of Fig.1) (Ishikawa et al., 2000). Although *B. brizantha* is relatively less adapted than *B.decumbens* to infertile acid soils, *B. brizantha* was found to be superior in its level of Al resistance to the other crops tested.

Fig. 1. Differences in Al resistance among crops and *Brachiaria brizantha*. The left panel shows Al resistance values in long-term experiment and short-term experiment. The right figure shows the relationship between Al resistance in long-term experiment and that in short-term experiment. ① *B. brizantha*, ② rice, ③ tea, ④ maize, ⑤ pea, ⑥ siclepod, ⑦ barley

We also compared nutrient acquisition ability of *B. brizantha* with that of crops. Nutrient acquisition ability was compared by quantifying relative nutrient status in shoots in Al treatment to that in low pH • low P treatment. Among the 5 species compared, *B. brizantha* was found to have superior nutrient acquisition abilities for K,P and Mg (Fig. 2). However, *B. brizantha* showed the lowest acquisition ability for Ca. *B. brizantha* can acquire highest amounts of K, P and Mg even under high Al, low P and low pH conditions. *B. brizantha* may have the lowest requirement of Ca for normal growth even in acid soil conditions. This greater ability of *B. brizantha* to acquire nutrients under simulated acid soil conditions could

Fig. 2. Relative nutrient status of each nutrient concentration in Al treatment to that in control treatment

be responsible for its greater vigour in acid soil conditions during the pasture establishment phase (Rao et al., 1996).

2.1.2 Differences in Al resistance, Al accumulation and plasma membrane permeability among brachiariagrasses

Adaptive responses of several brachiariagrasses to infertile acid soils have been identified and described by previous research (Louw-Gaume et al., 2010 a,b; Rao et al., 1995, 1996; Wenzl et al., 2001, 2003). We compared Al resistance, Al accumulation (hematoxylin staining method [Wagatsuma et al., 1995] and PM permeability (FDA-PI fluorescence staining method [Ishikawa et al., 2001]) among 4 brachiariagrasses in short-term experimental conditions that were described in the former section, together with most Al-resistant rice cultivar Rikuu-132 (Khan et al., 2009) as a reference plant species. Al resistance was ranked as follows: *B. decumbens*, *B. hybrid*, *B. brizantha* > *B. ruziziensis* > Rikuu-132 (Fig. 3). Al resistance of *B. hybrid* (cv. Mulato) and *B. brizantha* was found to be comparable to that of *B. decumbens* which has been ranked as the most Al-resistant brachiariagrass (Wenzl et al., 2001). Although Al resistance of *B. ruziziensis* was found to be markedly lower than *B. decumbens* (Wenzl et al., 2001, 2003), its resistance level was higher than that of the most Al-resistant rice cultivar. It was suggested that the highest Al resistant phenotype of *B. hybrid* may be ascribed to the Al resistance genes from *B. decumbens* or *B. brizantha* and not from *B. ruziziensis*. Al accumulation was localized mainly within 1- mm root-tip portion and its concentration corresponds reversely to Al resistance order: the least Al accumulation was recognized for the most Al-resistant *B. decumbens*. PM lipid layer was less permeable to Al in brachiariagrasses than in the most Al-resistant rice cultivar and its less permeable PM characteristic was localized mainly within 1- mm root-tip portion (Fig.4).

Fig. 3. Differences in Al resistance among brachiariagrasses and Al-resistant rice cultivar Rikuu-132

Fig. 4. Differences in Al accumulation and PM permeability among brachiariagrasses and Al-resistant rice cultivar (Rikuu-132)

Permeability of PM was negatively associated with Al resistance: the least PM permeabilization was observed with the most Al-resistant *B. decumbens*. The less permeability of PM and the lower Al accumulation in root-tip portion in Al-resistant brachiariagrass agree well with the former results which have been recognized in Al-resistant plant species, cultivars, or lines (Ishikawa et al., 2001; Ishikawa & Wagatsuma, 1998; Wagatsuma et al., 2005).

2.1.3 Lipid composition and phenolics concentration in root-tip portion of brachiariagrasses in relation to Al resistance

The lower ratio of PL to sterols (S) (PL was measured by molybdenum blue spectrophotometric method after extraction with isopropanol-chloroform- H_2O [2:2:1] ; S was measured by ortho-phthalaldehyde colorimetric method after extraction with dichloromethane-methanol [2:1] [Khan et al., 2009]) in root-tip portion was found to be beneficial for the less permeability of PM in the presence of Al, which agrees with the results of rice cultivars (Khan et al., 2009). In the more proximal root region (0-10 mm from root apex), the ratio of PL to S for *B. decumbens* was higher than that of *B. ruziziensis*, but on the contrary, it was lower in root-tip portion (0-2 mm from root apex) under Al treatment conditions (Fig.5).

The lower negativity of PM surface that was associated with the lower ratio of PL to S in root-tip portion could contribute to lower permeability of PM to Al. This is highly consistent with Gouy-Chapman-Stern model of Al rhizotoxicity (Kinraide, 1999). In case of rice cultivars (Khan et al., 2009), wheat lines, triticale lines, maize cultivars (unpublished data),

lipid compositional difference in connection with Al resistance were recognized in root-tip portion of 0-10 from root apex. However, in brachiariagrasses, lipid compositional difference was related with Al resistance only in root-tip portion of 0-2 mm from root apex. We suggest that the high level of Al resistance in brachiariagrass is extremely localized at the root tip.

Fig. 5. Sterol or phospholipid content in the different segment of root of brachiariagrasses treated with or without Al in solution

It is known that phenolic compounds can be solubilized into lipid layer, and the lipid layer solubilized with phenolic compounds is transformed into the less fluid layer (Arora et al., 2000; Boija & Johansson, 2006). Higher concentration of phenolic compounds was detected in root-tip portion (0-5 mm and 0-2 mm from root apex) of *B. decumbens* than in *B. ruziziensis* (Fig.6).

The concentration of phenolic compounds was lower in the portion of 0-10 mm from root apex than that of the shorter part from root apex (data not shown). Phenolic compounds have been detected basically in the cell wall, vacuole, and to a small extent in the cytoplasm and nucleus (Hutzler et al., 1998). At around neutral pH of cytosol, the binding affinity to Al ions was significantly higher for phenolic compounds than for organic acids (Ofei-Manu et al., 2001). Higher concentration of phenolic compounds is considered to be more effective for greater detoxification of Al ions in cytosol of *B. decumbens*. Additionally, higher inclusion of phenolic compounds into PM lipid layer may be more favourable for making the PM less permeable in the presence of Al ions, although there are no reports on the inclusion of phenolic compounds in plant lipid layer. Several quantitative and qualitative changes in PM may contribute to superior level of Al resistance in *B. decumbens*. These include: higher proportion of S relative to PL, higher concentration of phenolic compounds in cytosol, and higher inclusion of phenolic compounds in PM lipid layer in root-tip portion. These changes

may contribute to an extremely strong PM lipid layer which plays a key role in exclusion of Al and high level of Al resistance in *B. decumbens*. Direct demonstration of the existence of phenolic compounds in PM lipid layer will be an important task for the future research.

Fig. 6. Total phenolic compounds in root-tips of two brachiariagrasses treated with or without Al in solution

2.1.4 Mechanisms of high level of Al resistance in *B. hybrid* (cv. Mulato)

B. hybrid showed higher resistance to Al similar to *B. decumbens*. When *B. hybrid* seedlings were grown with an extremely high concentration of Al (0.37 mM) for 10 days, no growth inhibition was observed (Fig. 7). Moreover, Al application did not inhibit the uptake of nitrogen (N), P and K in *B. hybrid*. *Andropogon gayanus*, a poaceous pasture grass, is also very resistant to Al and Al application significantly increased Al concentration in both leaf and root of this species (Fig. 8). In *B. hybrid*, by contrast, significant increase in Al accumulation was also observed in root but not in leaf. This indicates that some mechanisms restricting Al translocation from roots to shoots should exist in *B. hybrid*. The ^{27}Al NMR spectrum obtained from intact roots showed several peaks downfield at 10-20 ppm (Fig. 9a), suggesting that most of the soluble Al in roots makes complexes presumably with organic acid anions (Fatemi et al., 1992; Kerven et al., 1995). Since the ^{27}Al NMR spectrum did not change after removing soluble and/or loosely bound apoplastic Al, these Al complexes in roots were likely to be localized in the symplast of cells. In many Al-accumulator species, leaves and roots with high concentration of Al are detoxified by organic ligands, such as Al-oxalate in *Melastoma malabathricum* (Watanabe et al., 1998, 2005). The same mechanisms are considered possible in roots of *B. hybrid*. It has been reported that Cd translocation from roots to shoots is restricted by Cd isolation in root vacuoles (Miyadate et al., 2010). Al in the *B. hybrid* may also compartmentalize in root vacuoles and, thus, may not be translocated to shoots.

Fig. 7. Effects of Al toxicity on growth of *Brachiaria* hybrid, *Andropogon gayanus*, and barley. Growth was expressed as the relative dry matter accumulation (i.e. [dry weight after treatment - initial dry weight in each treatment]/[dry weight after treatment-initial dry weight in control treatment])

Fig. 8. Concetration of Al in *Brachiaria* hybrid, *Andropogon gayanus*, and barley after the Al treatment

Fig. 9. ^{27}Al NMR spectra in intact roots (a) and roots after removing soluble and/or loosely bound apoplastic Al by 0.1 M HCl (b). AlCl_3 (0.1 mM) was used as an external reference to calibrate the chemical shift (0 ppm)

2.2 Mechanisms of low P tolerance in brachiariagrass comparing with those in rice

2.2.1 Low P tolerance of *B. hybrid*

As indicated above, *Brachiaria* species are well adapted to the low-fertility acid soils of the tropics because they are highly tolerant of high Al and low supplies of P and Ca (Louw-Gaume et al., 2010b; Rao et al., 1995, 1996, 2001b; Wenzl et al., 2001). They have lower internal requirements for P than other grasses because they are able not only to acquire P with their extensive root systems but also to use the acquired P more efficiently for growth and metabolism (Rao et al., 1996, 1999). However, mechanisms of P-use efficiency are relatively less known in plants, including *B. hybrid*. Because carbon metabolism is well known to be affected by the P status in plant tissue (Rao, 1996), we studied low-P-tolerance mechanisms, in terms of P recycling and carbon metabolism, in the *B. hybrid* comparing them with those of rice (Nanamori et al., 2004).

B. hybrid and rice plants were cultivated in nutrient solutions with or without $32\ \mu\text{M}$ P. The data obtained on growth parameters and nutrient status are shown in Fig. 10. When P supply in the nutrient solution was low, root:shoot ratio increased, especially in the *B. hybrid*. We found that, for the *B. hybrid*, vigorous root growth is a mechanism for acquiring larger amounts of P from low P conditions. This finding was supported by the high levels of N concentration found in *B. hybrid* roots, while P concentration in *B. hybrid* leaves was significantly lower than that of rice leaves. Lower P concentration in *B. hybrid* leaves may indicate that the *B. hybrid* uses P more efficiently to sustain active metabolism for dry matter production. The P concentration of *B. hybrid* was quite low (0.44 and 0.56 mg-P/gDW in roots and leaves, respectively) and less than rice, which is also known as a low P tolerant plant. Results on the fractionation of P compounds indicated that acid-soluble P_i accounted for about half of the total P in the *B. hybrid* (Fig. 11). Results on the P_i :total P ratio in *B. hybrid* leaves under P deficiency indicate that the *B. hybrid* can survive with extreme low intracellular P_i concentration. This may be due to rapid turnover of other organic P pools under P-deficient conditions.

Chapin and Bielecki (1982) studied the impact of mild P stress on P fractions in relation to plant growth in barley and low-P-adapted barleygrass. They found that barleygrass had a higher proportion of Pi at each level of P supply. They explained this as a consequence of slower growth in barleygrass and higher P status rather than any inherent difference in mechanism. However, in our study, the higher Pi proportion in the *B. hybrid*, compared with that of rice, coincided with lower P concentrations, as explained above. We, therefore, speculate that recycling of internal organic P compounds could be an important mechanism of P-use efficiency in the *B. hybrid*.

Bosse and Köck (1998) have shown activities of APase and RNase were induced during P deficiency, and that this induction is associated with P turnover in plants. In our study, APase and RNase activities were both strongly induced in both rice and *B. hybrid* by P deficiency (Fig. 12). Induction of APase activity was markedly higher in roots under P-deficient conditions. Duff et al. (1994) reported the existence of extracellular APase in roots, where it is localized mainly in apical meristems and outer and surface cells. It is involved in hydrolyzing and mobilizing Pi from organic phosphates in the soil for plant nutrition. The induction of APase in roots may also be associated with excretion. Bosse and Köck (1998) suggested that the increase in activity of phosphohydrolases was a specific response to the decline of cellular available Pi in Pi-starved tomato seedlings. Although Pi in roots was lower than in shoots of both test crops, it was impossible to account for the difference of APase induction between roots and shoots only by the difference in intracellular Pi concentration. Thus, we suggest that some other signal transduction pathway must be operating between roots and shoots against P starvation in the cell. APase activity in shoots was greater in the *B. hybrid* than in rice, suggesting the possibility of rapid P turnover in the *B. hybrid*. This may enable the *B. hybrid* to survive under low P conditions. APase may not be a major mechanism for scavenging or acquiring P because differences in APase induction could not sufficiently account for the diverse growth response of genotypes of both common

Fig. 10. Growth (A) and P concentration (B) of rice and *Brachiaria* hybrid plants grown under P sufficient and deficient conditions. Error bars indicate S.E. (n = 3)

bean and maize plants under P deficiency (Yan et al., 2001; Yun & Kaeppeler, 2001). However, we observed in our study that APase activity was induced by P deficiency and the activity seems to be correlated well with P-use efficiency, as indicated by the lower value of total P concentration, so that the function of APase in adaptation to low-P conditions should not be underestimated. RNase activity was also high in roots under P-deficient conditions (Fig. 12). Nürnberger et al. (1990) and Löffler et al. (1992) showed that both extracellular and intracellular RNase activities were induced in tomato-cell culture under P deficiency. Extracellular RNase could help degrade the RNA from senescing cells that have been either damaged or lysed, and also help degrade any RNA that might be present in the rhizosphere. Thus, the high RNase activity in roots may be associated with secretion similar to APase. RNase activity in shoots was also greater in the *B. hybrid* than in rice, indicating that RNase also contributes to rapid P turnover. Glund et al. (1990) showed that, in the relationship between P_i concentration and RNase activity, induction of RNase under P starvation occurs when the intracellular content of P is very high.

Fig. 11. P_i concentration (A) and ratio of P_i to total P (B) of rice and *Brachiaria* hybrid plants grown under P sufficient and deficient conditions. Error bars indicate S.E. ($n=3$)

The above studies indicate that phosphohydrolases, such as APase and RNase, were induced by P deficiency as a P-recycling system. Coinciding with such a mechanism, it is possible that carbon metabolism could also be altered under P deficiency. We therefore studied photosynthate partitioning under P deficiency, tracing photosynthetically fixed ^{14}C in leaves. In rice, photosynthates mainly distributed to sugars, which consist of sucrose, indicating that rice enhanced the sucrose synthesis pathway (Fig. 13). The mRNA accumulation of sucrose phosphate synthase (SPS) also increased as mentioned previously. Hence, sucrose concentration in rice leaves was remarkably high (Fig. 13). The ^{14}C distribution proportion to sugars increased with P deficiency. Enhanced sucrose synthesis in rice leaves through P deficiency may contribute to P recycling because P is liberated during sucrose synthesis (Rao, 1996). However, sucrose catabolism was restricted because the ^{14}C distribution ratio to amino acids and organic acids decreased with P deficiency and with

Fig. 12. Acid phosphatase (A) and ribonuclease (B) activities in leaves of rice and *Brachiaria* hybrid grown under P sufficient and deficient conditions. Error bars indicate S.E. (n = 3)

carbohydrate accumulation (Fig. 13). Sucrose synthesis may, therefore, not contribute efficiently to P recycling. However, the ^{14}C distribution proportion to sugars in the *B.* hybrid was not as marked as in rice (Fig. 13), and the effect of P deficiency was smaller. The ^{14}C distribution ratio to amino acids and organic acids in the *B.* hybrid was greater than in rice, and slightly affected by P deficiency. The decrease of total organic acids and carbohydrates in *B.* hybrid leaves under P deficiency suggests that the *B.* hybrid can sustain active amino acid and organic acid pathways with enhanced sugar catabolism, using P efficiently under P deficiency. PK and its bypassing enzymes catalyze the PEP-consuming reaction in leaves, with PEPP activity increasing by a factor of 5.6 to 6.0 with P deficiency. This induction of PEPP is likely to be associated with P recycling, as Duff et al. (1989) suggested. PK was also induced by P deficiency, but not significantly in the *B.* hybrid. PEPC activity was slightly induced by P deficiency in rice but not in the *B.* hybrid. The decrease of PEPC activity in *B.* hybrid leaves would result from reduced net photosynthesis under P deficiency. Kondracka and Rychter (1997) suggest that facilitating the PEP metabolism may be important in view of the P recycle. PEPC and PEPP are considered to function in P recycling as PK-bypass pathways. If these enzyme activities are induced in P recycling, then the carbon flow to the TCA cycle is expected to increase. The ^{14}C distribution ratio to amino acids and organic acids increased slightly in the *B.* hybrid with P deficiency (Fig. 13), indicating that these bypassing enzymes may function to facilitate carbon flow to the TCA cycle. However, in rice, the ^{14}C distribution ratio to amino acids and organic acids decreased with P deficiency. Therefore, the PK bypassing mechanism under P deficiency may not contribute to facilitating the carbon flow to the TCA cycle in rice. In addition to the PK-bypassing mechanism, carbon export from chloroplast to cytosol via the triose-phosphate translocator (TPT) may be a process that significantly affects carbon partitioning under P deficiency (Rao, 1996). When plants are starved for P, triose-P exports from chloroplast to cytosol via TPT, and subsequent sucrose synthesis in the cytosol is likely to be restricted (Rao, 1996). The ^{14}C distribution ratio to sugars and to residue, which mainly consists of sucrose and

starch respectively, increased with P deficiency in both rice and *B. hybrid* (Fig. 13), indicating that restriction of triose-P exports from chloroplast to cytosol via TPT may not occur.

Fig. 13. Photosynthetically assimilated ^{14}C distribution (A), sucrose and starch concentration (B and C, respectively) in leaves of rice and *Brachiaria* hybrid. Error bars indicate S.E. ($n = 3$)

2.2.2 Transcriptomic analysis of P deficient rice plants

Rice (*Oryza sativa* L. ssp. japonica) plants were germinated and cultured in nutrient solutions containing 0 and 32 μM NaH_2PO_4 for -P and +P treatments, respectively. The seedlings were cultivated for 9 days after transplanting. Total RNA of leaves and roots was used for transcriptomic analyzes by using cDNA arrays (Wasaki et al., 2003b, 2006). As the response of rice roots, there were 15 up-regulated genes in the short-term (24 h) and 86 in the long-term (9 d) treatment with -P, whereas there were 23 and 97 down-regulated genes in the two treatments, respectively. The number of genes regulated (especially down-regulated genes) by the P deficiency was lower in leaves than in roots. There was one up-regulated gene in the short-term (24 h) and 48 genes in the long-term (9 days) -P treatments, whereas there were eight and four down-regulated genes in these two treatments, respectively. None

of the genes were regulated in a similar manner between the short and long-term -P leaves. This result suggests that the responses in P-deficient rice leaves are different between short- and long-term treatments, whereas those of roots are relatively similar. *OsPI1* (*Oryza sativa* phosphate-limitation inducible gene 1; Wasaki et al. 2003c), showed the most significant increase in its transcription in the long-term -P treatment, in both the roots and leaves. This gene was classified as a member of *TPS11/Mt4* family, which is the P-deficient responsive non-coding RNA. The *SqdX*-like gene, a homolog of sulfoquinovosyl diacylglycerol (SQDG) synthesis related genes, was up-regulated significantly in the -P roots. P deficiency enhances dynamic lipid reconstruction and causes SQDG or galactolipids accumulation and expression of a related gene in leaves (Essigmann et al., 1998; Nakamura et al., 2009). Because SQDG has the ability to substitute for PL, it was suggested that the increase of SQDG synthesis is available for the efficient use of P in the membrane (Essigmann et al., 1998). Four genes related to P metabolism were induced in leaves by long-term -P treatment. Inorganic pyrophosphatase and a phosphatase probably contributed to the maintenance of Pi concentration in the tissue by the direct production of Pi from organic phosphate compounds. It was concluded that the function of inorganic pyrophosphatase was common in both roots and leaves, because expression was induced in both organs by long-term -P treatment. Both bi-functional nuclease and S-like RNase expression levels were increased by the -P conditions; their contribution is to produce monomeric nucleotides as substrates for phosphatases (Duff et al., 1994; Green, 1994; Palma et al., 2000).

Many genes involved in polysaccharide metabolisms were up- and down-regulated in leaves by long-term -P and P re-supply treatments, respectively. It is probable that the up-regulation of ADP-glucose pyrophosphorylase, which is a key enzyme of starch synthesis, and starch synthetic enzymes such as starch branching enzyme and starch synthases, induces the accumulation of starch in leaves under -P conditions. In fact, there are many reports of the accumulation of starch in the chloroplasts of P-deficient rice and other plants (Ciereszko & Barbachowska, 2000; Fredeen et al., 1989; Nomura et al., 1995; Qui and Israel, 1992; Rao et al., 1993; Usuda & Shimogawara, 1991). We concluded that the starch accumulation in leaves grown under P-deficient conditions was caused by the disruption of the export of triose phosphate from the stroma by the Pi translocator (Nátr, 1992). Nátr (1992) also noticed the liberation of Pi by the enhancement of starch synthesis. Because starch synthesis and the induction of Pi utilizing enzymes are synchronized, it is a reasonable speculation that the starch accumulation in the P-deficient leaves is a result of the maintenance of the internal Pi concentration.

Fig. 14 shows a summary of metabolic changes based on the regulation of gene expression in the leaves and roots of rice exposed to -P stress. Some important metabolic changes in roots by -P are suggested, namely: (1) acceleration of carbon supply for organic acid synthesis through glycolysis; (2) alteration of lipid metabolism; (3) rearrangement of compounds for cell wall; and (4) changes of gene expression related to the response for metallic elements such as Al, Fe and Zn. The major responses in leaves were involved in internal P utilization. The response in leaves seems to be less dramatic than that in roots; however, it is probable that an important function is regulated in shoots, such as the regulation of the novel *TPS11/Mt4* gene family (Burleigh & Harrison, 1999), which contains rice *OsPI1* (Wasaki et al., 2003c).

2.2.3 Bypass pathways in rice for P use efficiency in plant

From our previous study using microarray on P deficient rice, we found that several genes relating C and P metabolism in chloroplast changed their expression level. One of them is phosphoenolpyruvate/phosphate translocator (PPT), and it showed enhancement under phosphorus deficient condition. PPT transports PEP into the chloroplast and antiports P_i to cytosol (Hausler et al., 2000), the role of PPT under P deficient condition is considered to supply substrate for the shikimate pathway. There exit another phosphate transporter; triose phosphate translocater (TPT) on chloroplast membrane which loads triose phosphate into cytoplasm and antiports phosphate into chloroplast. These two phosphate translocators are considered to regulate the phosphate level in the chloroplast.

Fig. 14. Summary of plant responses to phosphorus deficiency in shoot and root tissue

As comprehensive analysis of each pathway using intact plant has not reported, we evaluated it by using quantitative real time PCR (qRT-PCR) to determine the expression level of each gene under P deficient condition (Shinano et al., 2005). While the expression level of mRNA is not simply representing the activity of those enzymes corresponding to the gene, obtained information will be very useful to consider plant response to P deficiency.

2.2.4 Gene coding key enzyme of sucrose synthesis

The synthesis of sucrose will liberate phosphate from intermediate compounds, thus it is expected that the level of mRNA for SPS increased with -P treatment. SPS exist in cytosol of mesophyll cell and the combined reaction of SPS and sucrose phosphate phosphatase is main route for sucrose synthesis. That is, during sucrose synthesis, one molecule of Pi is liberated from sucrose phosphate. The -P treated plants first uses Pi stored in vacuole but after they used up all Pi in vacuole, cytosolic Pi content became lower. Then the plants with lower Pi concentration may facilitate sucrose synthesis and excrete Pi from sugar phosphate to keep up the Pi concentration in cytosol of mesophyll cell. Sucrose content in phosphate starved plant varies with species. In common bean and sugar beet, leaf sucrose content increased by P deficiency (Ciereszko & Barbachowska, 2000; Rao et al., 1990), although in leaves of *Arabidopsis* it decreased. Our results indicate that rice increases sucrose synthesis with P deficiency.

2.2.5 Genes coding candidates for glycolytic bypass enzymes

NADP dependent glyceraldehyde 3-phosphate dehydrogenase (NADP-G3PDH) instead of NAD dependent G3PDH (NAD-G3PDH), and PEPC instead of PK are expected to play alternative pathways to regulate carbon flow under P deficient condition.

In rice leaves under P deprivation, we did not see any increase in relative expression of NADP-G3PDH, which is known as P starvation inducible bypassing enzyme for NAD-G3PDH in *Brassica nigra* (Duff et al., 1989). On the contrary, NAD-G3PDH relative expression was significantly high in -P plants at 21 days. In the level of gene expression argument, this result may suggest NADP-G3PDH is not working as glycolytic bypass in rice plant. The lack of induction of nonphosphorylating pathway was also seen in other plant species, such as *S. minutum* (Theodorou et al., 1991) and *A. brevipes* (Guerrini et al., 2000). Also PEPC and PK have the relationships of glycolytic bypass induced under P deficiency (Li & Ashiharam, 1990). Even though PEPC was thought to be catalyzed with the alternative pathway of PK under P deficiency (Li & Ashiharam, 1990), relative expression of both genes was increased by -P treatment. Increase of PEPC expression by P deficiency is also known in lupin.

2.2.6 Genes encoding chloroplast membrane transporters

Precise value of Pi concentration in cytosol and chloroplast is not known while it is suggested that the value is between 10 to 15 mM in cytosol (Mimura, 1999) and 20 to 35 mM in chloroplast (Diez & Heber, 1984). This indicates that higher requirement for maintaining Pi level in chloroplast rather than in cytosol, and low P condition is expected to increase the level of TPT and *in versa* in PPT. While the expression level of TPT was not changed by P deficient condition, the expression level of PPT increased dramatically. When one molecule of P is transported into chloroplast as PEP while exporting one Pi, the incorporated PEP is

decomposed in the chloroplast thereby having no net change in the P level of the chloroplast. We assumed that the role of PPT is increasing the PEP metabolism and makes a cycle from primary photosynthate synthesized in chloroplast and metabolized in cytosol with glycolysis then re-enter chloroplast with PEP then decomposed to release Pi in the chloroplast. From the analysis of rice microarray, PKp (plastid type PK) and shikimate kinase expression were enhanced under P deficiency. These results indicate physiological adaptation to incorporate PEP into chloroplast to support photosynthetic carbon flow and synthesis of secondary metabolic compounds. Recently, another type of phosphate transporter (PHT2; 1, which has high homology with Na⁺/Pi symporter of fungi) was reported (Versaw & Harrison, 2002). There is need to evaluate how these transporters are operated to regulate phosphate flux within these subcellular organs.

3. Conclusions

Brachiariagrasses are highly adapted to infertile acid soils, however, the physiological and biochemical mechanisms responsible for their superior adaptation have not yet been fully defined. This chapter summarizes the recent progress towards this objective. Comparative differences in Al resistance among 4 brachiariagrasses and 6 reference plant species were analyzed, and the following order of Al resistance was observed: *B. decumbens*, *B. hybrid*, *B. brizantha* > *B. ruziziensis* > rice (the most Al-resistant cultivar Rikuu-132) > tea (cv. Yabukita) > maize (cv. Pioneer 3352) > pea (cv. Kinusaya) > siclepod > barley (cv. Manriki). The order of Al tolerance in the short-term experiment with exposure to Al (1-h of 50 μ M AlCl₃ in 0.2 mM CaCl₂ followed by 24-h of Al-free 0.2 mM CaCl₂) was well correlated with that in the long-term exposure experiment (2 months of Al treatment with full nutrients) in spite of the differences in the treatment conditions, i.e., duration of Al treatment, Al concentration, composition of co-existing nutrients, and pH. Short-term Al resistance screening technique is accepted to be useful for the evaluation of Al resistance in spite of the simple composition of the treatment solution, considering the positive correlation data obtained formerly among 15 cultivars of sorghum, 10 cultivars of maize, and 8 cultivars of rice. Brachiariagrass showed greater abilities to acquire K, P and Mg, and to tolerate to lower concentration of Ca in shoots in the presence of high concentration of Al in the growing medium including low P at low pH conditions. The level of Al resistance of *B. hybrid* was ranked to be high as comparable to the most Al-resistant *B. decumbens*. It was suggested that the highest Al resistance phenotype of *B. hybrid* may be ascribed to the Al resistance genes from *B. decumbens* or *B. brizantha* but not from *B. ruziziensis*. Extremely high level of Al resistance found in *B. decumbens* was attributed to localized tip portion of less than 2 mm from root apex due to low amount of Al accumulation, low permeability of PM to Al, lower ratio of PL to S, and higher concentration of phenolic compounds in the tip portion of root as compared with other brachiariagrasses. Thus *B. decumbens* is considered to possess multiple physiological and biochemical mechanisms to resist high level of Al in soil solution, and its strategy may be extremely localized in the tip portion of the root apex. *B. hybrid* also exhibited good level of Al resistance. When an extremely high concentration of Al (0.37 mM) was included into the culture solution, significant increase in Al accumulation was observed only in root part. ²⁷Al NMR analysis suggested that the most part of Al in roots was likely to be localized in the cytosol of cells in organically complexed forms and this complexation may inhibit greater upward translocation of Al to shoots, which is beneficial to reduce Al toxicity in shoots.

Fig. 15. Summary of metabolic changes in leaves and roots with P deficiency: A. Rice; B *Brachiaria* hybrid. Red arrows indicate P deficiency inducible pathways.

Our study shows that tolerance of low P in both rice and *B. hybrid* involved marked differences in P recycling and carbon metabolism. We summarized the proposed P recycling mechanisms involved in carbon metabolism of rice and *B. hybrid* in Fig. 15. For rice, strategies for low-P tolerance include (1) decreased carbon flow to amino acids and organic acids, and decreased N concentration; and (2) improved partitioning of photosynthates to sucrose, combined with restricted sugar catabolism. For the *B. hybrid*, low-P tolerance involved two major strategies under P deficiency: (1) increasing the ability to use P efficiently by inducing APase and RNase in shoots; and (2) enhancing sugar catabolism and subsequent synthesis of amino acids and organic acids in leaves. Brachiariagrasses also showed greater abilities to acquire K, P and Mg, and to tolerate low concentration of Ca in shoots in the presence of high Al concentration in the growing medium.

In summary, studies on physiological and biochemical mechanism of adaptation of brachiariagrasses grown under simulated conditions of low fertility acid soils indicated their higher level of resistance to Al and tolerance to low supply of P and Ca. This was mainly attributed to their greater ability of Al complexation and Al localization in roots, less upward translocation of Al to shoot tissue, improved P utilization efficiency due to high PPT, and greater acquisition efficiency of K, P and Mg. Identifying the genes responsible for these superior traits of brachiariagrasses is a major objective for future research.

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Our dependence on soil, and our curiosity about it, is leading to the investigation of changes within soil processes. Furthermore, the diversity and dynamics of soil are enabling new discoveries and insights, which help us to understand the variations in soil processes. Consequently, this permits us to take the necessary measures for soil protection, thus promoting soil health. This book aims to provide an up-to-date account of the current state of knowledge in recent practices and assessments in soil science. Moreover, it presents a comprehensive evaluation of the effect of residue/waste application on soil properties and, further, on the mechanism of plant adaptation and plant growth. Interesting examples of simulation using various models dealing with carbon sequestration, ecosystem respiration, and soil landscape, etc. are demonstrated. The book also includes chapters on the analysis of areal data and geostatistics using different assessment methods. More recent developments in analytical techniques used to obtain answers to the various physical mechanisms, chemical, and biological processes in soil are also present.

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