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Environmental effects on physical properties of Geohumus and effects of its application on drought responses in maize

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Preface

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SUMMARY

Geohumus belongs to a new generation of soil melioration/hydrophilic polymers; however, evidence is limited with regard to both, the ability of Geohumus to store water in variable abiotic environments and the effects of Geohumus or other hydrophilic polymers on plant genotypes in response to drought condition. Therefore, this study aims at providing necessary and complementary information for improving Geohumus usage under field condition, and to improve our ecophysiological understanding of the interactions between Geohumus, plant genotype and the growing environment.

Three series of experiments were conducted to investigate (1) how abiotic factors affect the water holding capacity and restorability of Geohumus, (2) how the application of Geohumus affects plant morphological and physiological traits in response to different irrigation scenarios such as full irrigation, water deficit, and re-watering and (3) how the application of Geohumus in different soil types affects drought induced plant root-shoot communication.

Water holding capacity (WHC) and restorability of Geohumus in mL water g^{-1} was determined by immersing teabags with fresh and used Geohumus in prepared media under laboratory conditions. A greenhouse experiment was carried out in order to analyze morphological and physiological responses of the two maize cultivars Mikado and Companero to progressive drought or full irrigation (field capacity) as affected by Geohumus. To obtain in depth information on Geohumus-plant interactions, a split root system experiment was conducted as a tool to investigate hydraulic and non-hydraulic root-shoot communication of Mikado and Companero under full irrigation, partial root zone drying, and deficit irrigation.

Our results showed a negative correlation between salt concentration and water holding capacity (WHC) of Geohumus due to replacement of water molecules by ions at the polarized sites within the polymer chain (James and Richards 1986). Furthermore, salt types affected the WHC of Geohumus differently; in particular, multivalent ions were stronger impeding Geohumus compared to monovalent ions. Consequently, Geohumus application to sandy soil with base fertilizer application or to compost could not improve soil water content. However, split fertilizer application to sandy soil containing Geohumus led to a significantly improved soil moisture content indicating that timing and amount of fertilizer should be carefully considered under Geohumus application. Furthermore, for field applications the effect of climate needs to be considered, since the WHC of Geohumus increased with increasing temperature.

The preferential ion uptake of Geohumus could translate into competition with plant roots for nutrient uptake from soil solution. On the other hand, Geohumus can capture nutrients which might have been lost for plants due to drainage. We found indications of these positive effects since biomass and leaf area of Mikado and Companero maize genotypes were increased compared to soils without Geohumus.

Theoretically, polymers could release stored water to plants under drought stress; which in turn could inhibit or delay chemical signaling. However, our results showed increased concentrations of $[ABA]_{leaf}$ and $[ABA]_{xylem}$ of both Mikado and Companero grown in sandy soil with Geohumus in response to drought compared to treatments without Geohumus. This hormonal response was associated with larger leaf area and greater biomass resulting in a higher plant water demand due to its increased transpiration area while Geohumus did not improve soil water content significantly. On the other, hand root/shoot ratio, absolute root length and root biomass were decreased in plants grown with Geohumus. This suggests that plants grown with Geohumus under drought conditions could not extract water from deeper soil layers. The split root experiments showed that the larger leaf area of plants grown with Geohumus in combination with limited moisture content of sandy soil resulted in a stronger chemical root-shoot signal related to water stress. Regardless the increased $[ABA]_{xylem}$ which is associated with a reduction of stomatal conductance, Geohumus application could result in a decreased leaf water potential under partial root zone drying. Mikado grown with and without Geohumus, as a genotype potentially adapted to drought conditions, was able (1) to maintain its water potential under water limited conditions by penetrating roots into deeper soil layers (2) to delay the expression of physiological traits associated with drought, and (3) to maintain its shoot weight in contrast to Companero, a drought sensitive cultivar.

The presented results are of relevance for the improvement of our understanding of the impact of abiotic factors such as temperature, salt concentration, and salt types on the WHC of Geohumus and therefore will help to optimize the application of hydro-gels under field conditions. Beneficial traits of plant genotypes grown under Geohumus application were identified, which will be valuable for breeding and applied programs targeting at crop improvement in arid and sub-arid regions and areas vulnerable to climate change.

ZUSAMMENFASSUNG

Geohumus gehört zu einer neuen Generation von Bodenhilsstoffen / hydrophilen Polymeren. Dennoch sind weder die Fähigkeit von Geohumus oder anderen hydrophilen Polymeren zur Wasserspeicherung unter variablen abiotischen Bedingungen noch die Effekte auf pflanzliche Genotypen unter Trockenheit hinreichend belegt. Daher beabsichtigt die vorliegende Studie, die notwendigen und ergänzenden Informationen zur Verbesserung des Gebrauchs von Geohumus unter Feldbedingungen bereitzustellen und unser ökophysiologisches Verständnis der Interaktionen von Geohumus, pflanzlichen Genotypen und Wachstumsbedingungen zu verbessern.

Drei Versuchsreihen wurden durchgeführt, um zu untersuchen (1) wie abiotische Faktoren auf die Wasserhaltekapazität und Regenerierbarkeit von Geohumus wirken, (2) wie die Anwendung von Geohumus morphologische und physiologische Merkmale der Pflanze in Reaktion auf verschiedene Bewässerungsszenarios wie Vollbewässerung, Wasserdefizit und Wiederbewässerung beeinflusst und (3) wie die Anwendung von Geohumus die trockenheitsinduzierte Wurzel-Spross-Kommunikation in verschiedenen Bodentypen beeinflusst.

Zur Bestimmung der Wasserhaltekapazität und der Regenerierbarkeit von Geohumus in mL Wasser g^{-1} wurden unter Laborbedingungen mit frischem und gebrauchtem Geohumus befüllte Teebeutel in verschiedene Medien getaucht. Zur Untersuchung der morphologischen und physiologischen Reaktionen der beiden Maissorten Mikado und Companero auf Trockenheit und Vollbewässeurng (Feldkapazität) unter Einfluss von Geohumus wurde ein Gewächshausversuch durchgeführt. Für weitreichendere Information hinsichtlich der Interaktionen zwischen Pflanze und Geohumus, wurde ein Split-Wurzel-Versuch durchgeführt, um die hydraulische und biochemische Wurzel-Spross-Kommunikation von Mikado und Companero unter Vollbewässerung, partieller Wurzelzonentrocknung (PRD) und Mangelbewässerung zu untersuchen.

Unsere Ergebnisse zeigen eine negative Beziehung zwischen Salzkonzentration und Wasserhaltekapazität von Geohumus aufgrund des Austauschs von Wassermolekülen durch Ionen an den polaren Stellen der Polymerkette (James und Richards 1986). Weiterhin wurde die Wasserhaltekapazität von Geohumus von verschiedenen Salzen unterschiedlich beeinflusst. Im Besonderen zeigten multivalente Ionen eine stärkere

Hemmung von Geohumus als monovalente Ionen. Daraus folgend konnte die Anwenung von Geohumus in Sand mit basaler Düngung oder in Komposterde den Bodenwassergehalt nicht verbessern. Dennoch konnte bei Sand unter einer schrittweisen Düngergabe durch Geohumus der Bodenwassergehalt signifikant verbessert werden, was darauf verweist, dass die zeitliche Planung und die Düngermenge bei der Anwendung von Geohumus sorgfältig erwogen werden müssen.

Die bevorzugte Aufnahme von Ionen durch Geohumus kann zu einem Wettbewerb um Nährstoffe aus der Bodenlösung mit Wurzeln führen. Andererseits kann Geohumus Nährstoffe im Boden halten, die möglicherweise durch Perkolation für die Pflanze verloren gegangen wären. Eine höhere Biomasse und Blattfläche der Maisgenotypen Mikado und Companero unter Beimischung von Geohumus sind ein Beleg für diesen positiven Effekt.

Da Polymere theorisch das gespeicherte Wasser unter Trockenstress an die Pflanzen abgeben könnten, könnte im Gegenzug ein chemisches Signal unterdrückt oder verzögert werden. Dennoch zeigten unsere Ergebnisse höhere Konzentrationen von $[ABA]_{leaf}$ and $[ABA]_{xylem}$ sowohl in Mikado als auch in Companero in Sand mit Geohumus unter Trockenheit im Vergleich zu Behandlungen ohne Geohumus. Diese hormonelle Antwort war assoziiert mit höherer Blattfläche und Biomasse, was zu einem größeren pflanzlichen Wasserbedarf aufgrund einer größeren transpirierenden Oberfläche führte, während Geohumus den Bodenwassergehalt nicht signifikant verbessern konnte. Andererseits waren das Wurzel-Spross-Verhältnis, absolute Wurzellänge und Biomasse der Wurzel bei Pflanzen mit Geohumus erniedrigt. Dies legt nahe, dass Pflanzen mit Geohumus unter Trockenheit keinen Zugang zu Wasser in tieferen Bodenschichten haben. Die Split-Wurzel-Versuche zeigten, dass eine größere Blattfläche nach Geohumusapplikation in Kombination mit limitierter Bodenfeuchte bei Sand in einem stärkeren mit Wasserstress assoziierten chemischen Wurzel-Spross Signal resultierte. Trotz der höheren Konzentration $[ABA]_{xylem}$, welche mit einer Reduktion der stomatären Leitfähigkeit assoziiert ist, konnte die Anwendung von Geohumus zu einem reduzierten Blattwasserpotential unter partieller Wurzelzonentrocknung (PRD) führen. Mit oder ohne Geohumus konnte Mikado, als potentiell an trockene Bedingungen adaptierter Genotyp, (1) sein Wasserpotential unter wasser-limitierten Bedingungen durch Durchwurzelung tieferer Bodenschichten aufrechterhalten um (2) die Ausbildung mit Trockenheit assoziierter Merkmale zu verzögern und (3) das Gewicht des Sprosses im Gegensatz zu Companero, einer gegenüber Trockenheit sensitiven Sorte, beizubehalten.

Die dargestellten Ergebnisse sind für die Verbesserung unseres Wissens über den Einfluss von abiotischen Faktoren wie Temperatur, Salzkonzentration und Salzart auf die Wasserhaltekapazität von Geohumus von Bedeutung und werden daher bei der Optimierung der Anwendung von Hydrogelen unter Feldbedingungen beitragen. Nützliche Eigenschaften von pflanzlichen Genotypen unter der Anwendung von Geohumus wurden identifiziert. Dies wird sowohl in der Pflanzenzüchtung als auch bei angewandten Programmen zur Verbesserung von Nutzpflanzen in ariden und semi-ariden Regionen und vom Klimawandel bedrohten Gebieten von großem Nutzen sein.

INTRODUCTION

The crucial challenge for the future is securing food production for future generations. Until 2050, world population is estimated to grow by 3.7 billion (Wallace 2000). At the same time, up to 90% of freshwater required may be affected by climate change (Morison, Baker et al. 2008). Among the effects of climate change are increasing temperatures and altered precipitation patterns (IPCC 2007), which severely affect life in arid and semi-arid regions of the world (Lanen, Tallaksen et al. 2007), as the negative effects of extended drought periods encompass reduced production of food, resulting in food insecurity, especially in developing countries (Ceccarelli, Grando et al. 2007).

Drought is the most challenging stress threatening yields and constant efforts in research try improving crop productivity under water limited conditions (Cattivelli, Rizza et al. 2008). Some progress has been made for crop production under drought by improving soil and crop management, plant breeding, and biotechnology (Parry, Flexas et al. 2005). Additionally, the application of gel-forming or super-absorbent polymers, which have been applied since the early 1980's, have been shown to retain water under drought conditions (AMAS 1997). Literature shows that hydrophilic polymers have the potential for remarkable achievements in agricultural fields such as the increase and maintenance of water availability in soil (Johnson 1984), improving water use efficiency and survival of seedlings (Abedi-Koupai and Asadkazemi 2006; Dorraji, Golchin et al. 2010), enhancing plant nutrient uptake (Silberbush, Adar et al. 1993b; Mikkelsen 1994), and mitigating nutrient losses (Mikkelsen, Jr. et al. 1993). However, some results illustrated that water absorption of hydrophilic polymers was reduced by salt concentration (AI-Darby, Mustafa et al. 1990), type of ions (Foster and Keever 1990), and temperature (Andry, Yamamoto et al. 2009).

Similar to hydrophilic polymers, Geohumus is described as a new generation of soil melioration products. It is attributed to offer some typical advantages, such as increase of water use efficiency, reduction in need for irrigation, and stimulation of root growth.

However, up to now, evidence is limited with regard to both, the ability of Geohumus to store water in variable abiotic environments and the effects of Geohumus on plant growth performance under stress. The only study available (Trimborn, Heck et al. 2008) indicated that the application of Geohumus increased water use efficiency and biomass of sunflower, rape, maize, buckwheat, and cocksfoot under both drought and field capacity due to increased nitrogen uptake but surprisingly it could not improve plant water availability. It appears likely that Geohumus on the one hand improves plant growth and yields under a certain combination of environmental conditions and plant species. However, the benefits of applying this polymer could be lost or even change into negative effects in case abiotic factors such as temperature, salinity, or water availability modify the physical traits of the polymer. Further, no information is available about the role of different plant genotypes in combination with Geohumus under identical environmental conditions. Does the application of Geohumus require e.g. genotypes tolerant to drought or could this aspect be neglected when selecting the cultivar? Evidence related to the range of environments under which Geohumus will develop positive effects for plant growth will certainly contribute to the efficient use of this polymer in crop production systems. The aim of this thesis was therefore to collect more information with regard to the performance of Geohumus in controlled greenhouse and laboratory based experiments providing different combinations of environments, including drought scenarios, soil types, and plant genotypes. In depth analyses were carried out to address the following research questions:

(1) How are abiotic factors affecting the water holding capacity and restorability of Geohumus?

(2) How does the application of Geohumus affect plant morphological and physiological traits in response to different water scenarios such as full irrigation, water deficit, and re-watering?

(3) How does the application of Geohumus in different soil types affect drought induced plant root-shoot communication?

In a first series of experiments the water holding capacity (WHC) and restorability of Geohumus was analyzed as affected by temperature, immersion duration, different media (distilled water, tapwater, soil extract, compost extract, nutrient solution, and soil extract plus nutrient), concentration of nutrient solution, concentration and valance types of selected salts, soil incorporation depth.

A second series of experiments analyzed the effects of Geohumus on morphological and physiological responses of two maize cultivars (Mikado and Companero) under different water supply (full water supply, water deficits, prolonged drought, and re-watering after drought).

Parameters analyzed were non-hydraulic responses (pH_{xylem}, ($[ABA]_{xylem}$ and $[ABA]_{leaf}$), water status (leaf and root water potential and leaf and xylem osmotic potential), gas exchange (stomatal conductance, transpiration, and net photosynthesis), growth (leaf area, leaf weight, root weight, stem weight), biomass accumulation (root weight and distribution, root-shoot ratio, partitioning coefficient) and water use efficiency.

3) A third set of experiments was conducted to measure effects of Geohumus on drought induced root-shoot communication of the maize genotypes Mikado and Companero grown in split root system (SRS). In addition, soil type (sand and compost) was also included in this experiment because soil type could affect on genotypic response and Geohumus water holding capacity.

We analyzed water potential and osmotic potential of leaf and root, xylem pH, leaf and xylem, and stomatal conductance of two cultivars (Mikado and Companero) grown in split root system filled with sandy soil and compost with and without Geohumus under three water supply levels comprising full irrigation, partial rootzone drying, and deficit irrigation to respond to two following specific objectives e.g. effects of either soil type or Geohumus-soil type combinations on drought induced genotypic root-shoot communication.

1. STATE OF THE ART

1.1 Hydro absorbing polymers as soil amelioration tool

Water-absorbing Polymers were developed 20 years ago, and have been mainly applied to agricultural fields in arid climates, aiming to improve water absorption of soils and therefore irrigation efficiency (AMAS 1997). There are three major groups of hydrophilic polymers, depending on their original properties including natural polymers (proteins polysaccharides, lignin, and rubber), semi-synthetic (natural polymers combined petrochemicals), and synthetic polymers (vinyl and acrylic monomers) (Mikkelsen 1994). With regard to agricultural use, two main types of polymers consisting of soluble and insoluble components in water can be distinguished (AMAS 1997) (**Table 1.1**).

1) Water soluble polymers with primary products belonging to the semi-synthetic group (Mikkelsen 1994), were applied to aggregate and stabilize soil, prevent erosion and percolation. They include poly(ethylene glycol), poly(vinyl alcohol), polyacrylates, polyacrylamide, poly(vinyl acetate-alt-maleic anhydride) and have linear chain structures.

2) Water insoluble polymers, known as Gel-forming polymers, hydrogels or agricultural polymers, belong to the synthetic polymers (Mikkelsen 1994), which are characterized by a cross-linked structure to form a three dimensional network (AMAS 1997).

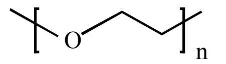
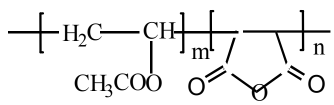
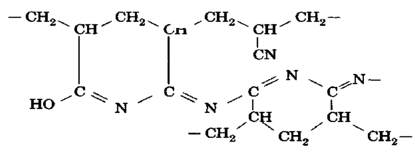
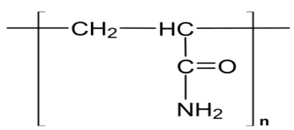
Agricultural polymers absorb significant quantities of water without dissolving, due to proper chemical cross-links that bind the polymer segments together (Mikkelsen 1994). Water insoluble agricultural polymers can be subdivided into three main polymer types (AMAS 1997)

(1) Starch-graft copolymers obtained by graft polymerisation of polyacrylonitrile onto starch followed by saponification of the acrylonitrile units

(2) Cross-linked polyacrylates

(3) Cross-linked polyacrylamides and cross-linked acrylamide-acrylate copolymers containing a major percentage of acrylamide units.

Table 1.1 Typical characteristics of agricultural polymers

Polymer form	Chemical name	Chain structure	Chain type	Application
Soluble	Poly(ethylene glycol)	 (*)	linear	Aggregating and stabilizing soil, preventing erosion and percolation
	Poly(vinyl acetate-alt-maleic anhydride)	 (*)		
Insoluble	Saponified Polyacrylonitrile Starch Graft Polymer	 (**)	Cross-linked	Increasing SM and WUE, and reducing fertilizer leaching and plant stress
	Polyacrylamides	 (***)		

Note: source of chain structure: (*) (AMAS 1997), (**) (Rodehed and Ranby 1986), and Wikipedia. WUE: water use efficiency, SM: soil moisture.

The chemical properties of the media to which they were applied (soil and water) also affected water absorption of agricultural polymers (Johnson 1984). Due to the chemical composition of polymers, they may further increase nutrient retention and exchange capacity of the soils to which they are applied, due to ionic components in their structure (Mikkelsen 1994). According to Kazanskii and Dubroveskii (1992) cited by (Mikkelsen 1994), polymers can absorb water because they were attached with polar groups such as hydroxyl, carboxyl or amino groups in their structures form a three dimensional network of macromolecule carbon chains, so that they can swell by absorbing up to 1000 times their own weight in water. **Fig. 1.1** shows the process of typical hydrophilic polymer's water absorption from the dry state to the water-swollen form. **Fig. 1.2** shows a microscope image with the expanded water saturated polyacrylamide structure of a polymer. Water molecules were absorbed by ionic groups of polymer chains under aqueous condition leading to expanded vacuoles of the polymers.

Geohumus, belonging to the synthetic polymers and non-soluble polyacrylate type, is known as a new generation of soil melioration products, which are is attributed to offer some typical advantages such as to improve plant water use efficiency, less frequent irrigation, and stimulation of root growth. Geohumus is made of 25% organic (cross-linked, partially neutralized polyacrylic substances) and 75% mineral components (ground rock, minerals and washed sand). Geohumus, theoretically, is able to absorb and store water up to 40- times its own weight.

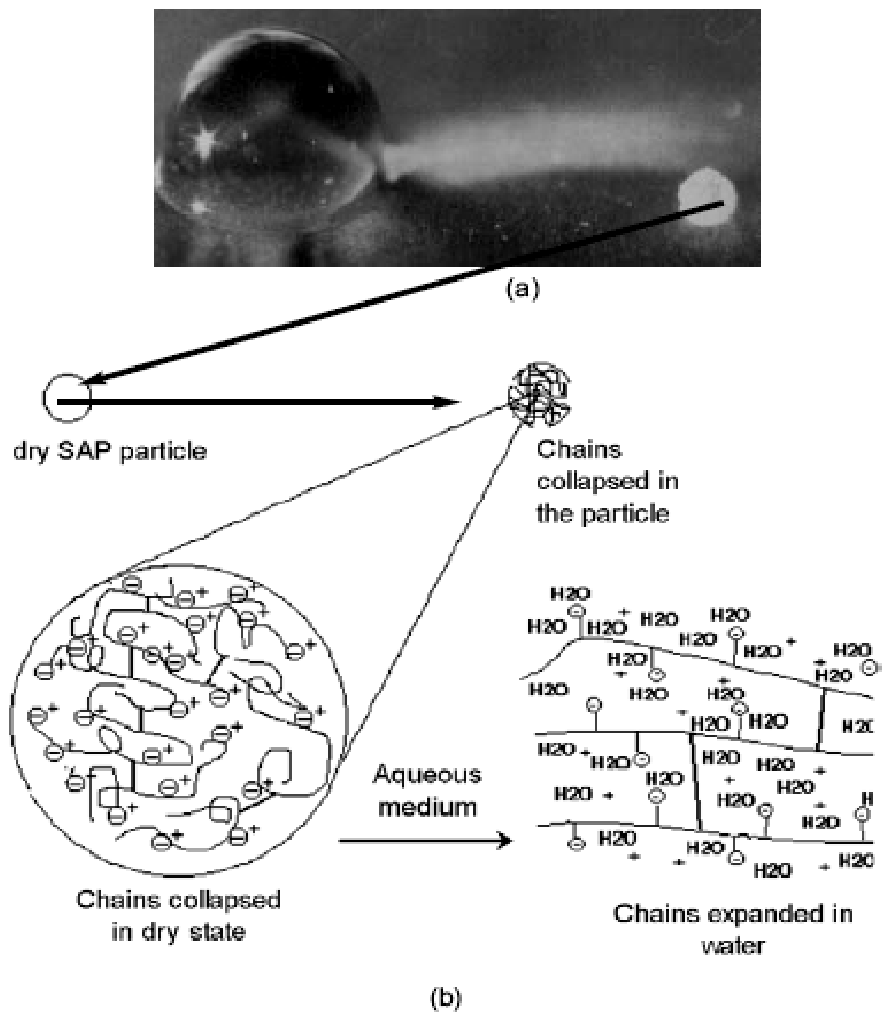


Fig. 1.1 Illustration of a typical acrylic-based anionic SAP material: (a) A visual comparison of the superabsorbent polymer (SAP) single particle in dry (right) and swollen state (left). The sample is a bead prepared from the inverse-suspension polymerization technique. (b) A schematic presentation of the SAP swelling (Zohuriaan-Mehr and Kabiri 2008).

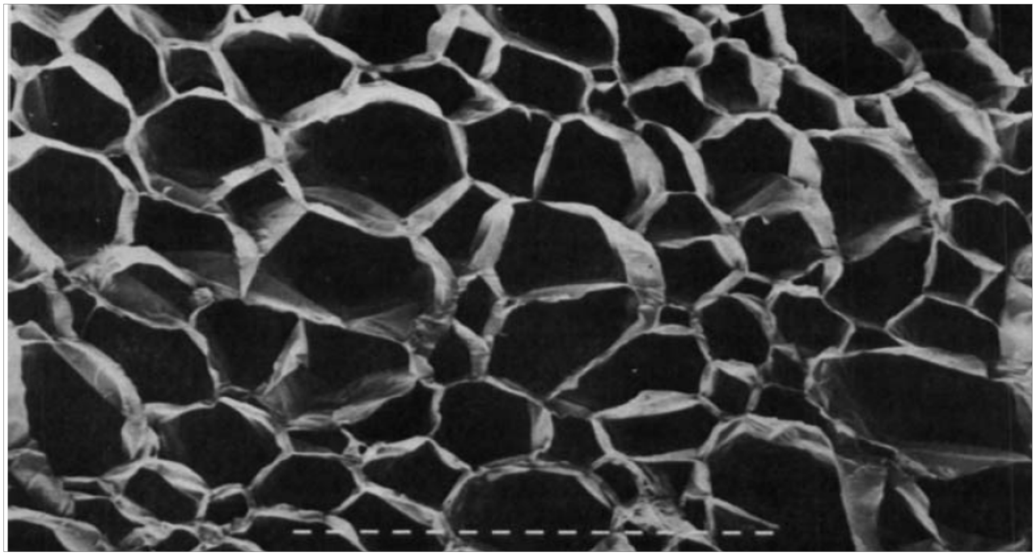
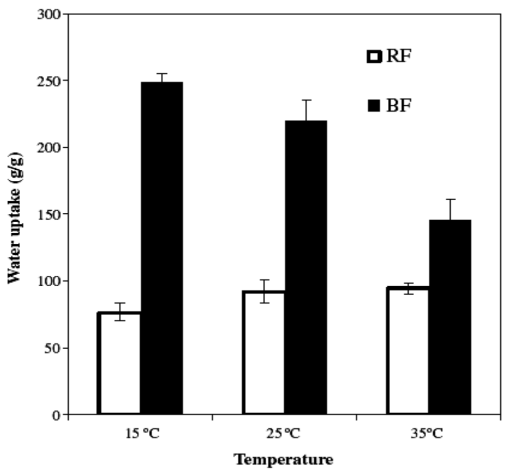


Fig. 1.2 View of the cut surface of expanded polyacrylamide (marker= 10pm) (Johnson and Veltkamp 1985).

1.2 The performance of Hydro-absorbing polymers

1.2.1 Effect of temperature on water absorption capacity

Results from earlier experiments (Andry, Yamamoto et al. 2009) illustrated that different polymers showed differences in water holding capacity under varying temperatures. For example, carboxylmethylcellulose (RF) increased water absorption at temperatures ranging from 15-35^{o}C, while isopropyl acrylamide (BF) expressed an opposite trend under the same temperature conditions **(Fig. 1.3)**. Differences in water absorption between these polymers depended on temperature. The decrease in absorbency of BF at temperatures above 'lower critical solution temperature', approximately 25-32^{o}C, could be due to the weakening of hydrogen bonds between the polymer's hydrophilic groups and water molecules with increasing temperatures. The peak of water absorption reaches its maximum at 50^{o}C and sharply decreases under higher temperatures **(Fig. 1.4)**. Above 50^{o}C molecular chains of polymers become shorter leading to declining molecular weight. Consequently, the network structure is constraint, so that water absorption capacity decreases (Suo, Qian et al. 2007). Below 50^{o}C, the polymerization ratio is higher leading to the reduction of cross-linking efficiency.



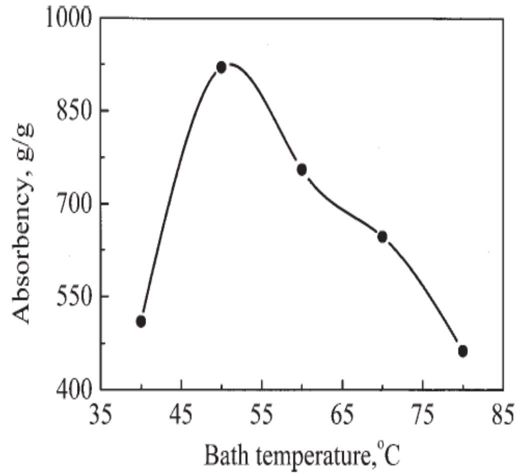


Fig. 1.3 Water absorption capacity of the absorbent alone under different temperature conditions. Bars indicate ±1 standard deviation. BF: isopropyl acrylamide and (RF): carboxylmethylcellulose (Andry, Yamamoto et al. 2009).

Fig. 1.4 Effect of the temperature on the water absorbency of Carboxymethyl Cellulose-graft-po(lyacrylic acid-co-acrylamide) (Suo, Qian et al. 2007).

1.2.2 Effects of salt concentration and valance types on water absorption of hydrophilic polymers

***Salt concentration**:* All gel-forming polymers reviewed here, including starch co-polymers, polyvinyalcohols as well as polyacrylamides, were affected by soluble salts, even when the solutions were classified as non-saline (Johnson 1984). The water absorption of gels is limited by several factors, such as cation types, valance number, and the concentration of nutrient solutions (Martin, Ruter et al. 1993). Several previous experiments illustrated that the electric conductivity of solutions was found to be the main factor affecting the swelling capacities of three hydro-absorbers, namely Sta Wet, Superhydro and hydrogel (AI-Darby, Mustafa et al. 1990), and Superab A200 (Dorraji, Golchin et al. 2010). Hydrogel amendments showed highest and fastest absorption rates in distilled water, but its water absorption capacity was limited in water with high salt concentrations (Akhter, Mahmood et al. 2004). Similarly, water holding capacity of cross-linked polyacrylamide in $CaCl_2$ solution sharply reduced with increasing EC (electronic conductivity) i.e. water available for plant decreased by 69 and 95% as imbedded in 2 and 4 dS m^{-1} $CaCl_2$ respectively and in NaCl solution with 4 dS m^{-1} comparing to distilled water (Green, Foster et al. 2004).

Polymers with different properties differ in water absorption behavior (Johnson 1984; AI-Darby, Mustafa et al. 1990; Smith and Harrison 1991) and consequently, polymers responded differently to solutions of $(NH_4)_2SO_4$ and KNO_3. Polyvinyalcohols could take up slightly more water than polyacrylates in a KNO_3 solution, but no difference was found in water uptake in $(NH_4)_2SO_4$ solution (Smith and Harrison 1991). However, specific polymers, such as starch co-polymers in high concentration solutions (20g N L^{-1} $(NH_4)_2SO_4$ and KNO_3 to saturation), showed stable water absorbing characteristics (Smith and Harrison 1991).

Previous result from Andry, Yamamoto et al. (2009) illustrated the interaction of polymers properties and salt concentration showing a close negative correlation between water absorption and salt concentration in BF whereas in RF this effect was much less pronounced.

Effect of ions on water absorption*:* The specific characteristics of the amendments also need to be considered as they contain various amounts and types of ions affecting the polymers in solution. Hydrated Micromax slowly releases $Fe^{2+,}$ Mn^{2+}, Zn $^{2+}$, and Cu^{2+} over a period of up to 18 months. Gypsum and dolomitic limestone release Ca^{2+} and Mg^{2+}, respectively. Osmocote 18N-2.6P-I0K (18-6-12) releases the cations NH_4^+, K $^+$ and Ca^{2+} over a 8- to 9-month period. Besides, in the same amendment, there were significant differences in water absorption that is attributed to their properties (Foster and Keever 1990)

The water retention capacity of hydrogels was reduced considerably in tap water (up to 30%) and nutrient solution (up to 75%) compared to distilled water. Water absorption levels depended on the type of hydrogel (TerraSorb: 141 times Hydreserve: 410 times). Besides, within the same type, coarse hydrogel, Austra-sorb, can retain almost twice the amount of water as fine ones; FeSO4 and NaFeEDTA differently affected on different polymers although they had the same concentration of iron (20 mg L^{-1} Fe) (Lamont and O'connell 1987).

Gu49® had no effect on water absorption of Igetage P and Terrasorb 200 (both of cross-linked polymers) which contain iron oxides and released only small amounts of free ions into solutions, whereas iron sulphate and Macromax responded in the opposite way. Sequestrene 138 with free iron species lead to strong impairment in water holding capacity of Igetage P and Terrasorb 200 (James and Richards 1986).

When estimating the effect of different solutions on water holding capacity of polymers, it is essential to consider the conductivity and valance of the elements in solution, as hydrophilic characteristics are affected by these two factors. In fact, divalent ions (Mg^{2+}, Ca^{2+}, SO_4^{2-})

reduce water holding capacity of polymers more severely than monovalent (Na^+, HCO_3^-, Cl^-) ions (Johnson 1984; Green, Foster et al. 2004).

The mechanism of ionic effects on polymers may be explained by the creation of ionic bonds between carboxyl groups inside the matrix of the gels, leading to a reduction in hydration by weakening electrical repulsion of aligned co-polymer chains and the structure of the gels determining selective ion absorption (Martin, Ruter et al. 1993). The presence of multivalent ions in the solution impeded the water absorption of hydrophilic gels by replacing and removing water at polarized sites on the surface of and within these hydrogels (James and Richards 1986).

1.2.3 Polymers' capacity in absorbing ions and release of nutrients

Depending on the degree of ionization in the chains of the polymers, their source and strength of charge leads to the exchange of selected ions in solution (Mikkelsen 1994) **(Table 1.2)**. Polymers differ in respect to nitrogen absorption. Smith and Harrison (1991) observed an interaction between polymers and ammonium ions in $(NH_4)_2SO_4$ solution. Starch co-polymers, polyvinyalcohols as well as polyacrylamides are negatively charged. This was of particular importance when starch co-polymers, polyvinyalcohols and polyacrylamides were imbedded in urea, ammonium, and potassium solutions (Smith and Harrison 1991).

Table 1.2 Some typical ionic groups in hydrophilic polymer chains (Mikkelsen 1994) adapted from Dyson (1987)

Source of charge	**Strength of charge**		
	weak	**Medium**	**Strong**
Anionic	Carboxylate – CO_2^-	Phosphate – PO_2H^-	Sulfonate – SO_3^-
Cationic	Amine – NH_3^+		Quanternary –NR_3^+

Incorporating hydrogels in saline soils led to a decreased concentration of Na^+ and Cl^- in the soil while the content of Ca^{2+} was increased. There was no impact of stockosorb K 410 on K^+ and Mg^{2+} in saline soils compared to the untreated control (Chen, Zommorodi et al. 2003). The results of X-ray microanalysis showed that Ca^{2+} was absorbed by the gel matrix at much higher rates than Na^+ as polymers can exchange cations. Additionally, these polymers, highly cross-linked polyacrylamides, possess more oxygen atoms, so the bonds between the polymer and Ca^{2+} are more stable than the bonds between polymers and Na^+ (Chen, Zommorodi et al. 2003). Ca, Zn, Mg, K, Fe, P, S and Mn were absorbed by polyacrylamide gels when these gels were imbedded in Hoagland's nutrient solution. The ion absorption of gels decreased from surface toward to center, while most of them absorbed at the surface (Martin, Ruter et al. 1993). Other polymers such as Stockosorb and Luquasorb could hold sodium and chloride from soil solution because their water holding capacities are high (Shi, Li et al. 2010).

Applying polymers to a soil may limit the release of nitrogen from dry fertilizer granules to the soil solution (Smith and Harrison 1991). The use of Igeta-green P, a hydrophilic gel, yielded positive effects by reducing the leaching of NH_4^+ , Ca^{2+}, Mg^{2+}, $Zn^{2+}$and K^+, whereas the leaching of NO_3^- was not reduced. This positive effect was less pronounced at higher concentrations of ammonium (Magalhaes, Wilcox et al. 1987). The gels ((Igeta-green P, a vinyl alcohol-acrylic acid copolymer sodium salt) with 0.2% mixed with soil improved N and P uptake but hindered Ca^{2+}, Mg^{2+} uptake of radish shoots while the iron content in roots increased with gel treatment (Magalhaes, Wilcox et al. 1987). Stockosorb K 410 mitigated adverse impacts of salinity on *Populus euphratica* in a saline soil by absorbing large amounts of water, leading to the dilution of Na^+ and Cl^- in the soil solution (Chen, Zommorodi et al. 2003). However, increasing the ratio of hydrophilic gel (Agrosoak) in a sandy soil reduced Cl^-, K^+, Ca^{2+} and Mg^{2+} but Na^+ and P accumulated in leaves of cabbage (*Brassica oleraceae L.*) (Silberbush, Adar et al. 1993a). In experiments carried out on maize (*Zea mays L.*), the concentration of Na, N, K in maize leaves increased as a function of increasing amounts of Agrosoak in the soil (Silberbush, Adar et al. 1993b).

The application of hydrophilic polymers in sandy soils showed positive effects on water holding capacity, water use efficiency, and in reducing the impact of salinity, as well as in increasing the yield of plants (Dorraji, Golchin et al. 2010). Mixing polymers with nutrient solutions prior to their application to soil can reduce the loss of N and K by leaching and increase nutrient uptake of plants (Mikkelsen 1994).

1.2.4 pH-effects on water absorption of hydrophilic polymers and retroaction

As mentioned above, water absorption of polymers depended on both salt concentration and type of ions of media. In addition, the pH value of the environment also affects the water absorption of polymers. Generally, the maximum water absorption capacity of polymers in soil is reached at pH 6.8 (Johnson 1984).

The effect of polymers on soil pH, as reported by Liu et al. (2006b) cited by (Bai, Zhang et al. 2010), depended on both the super-absorbent polymers and the soil characteristics. The results from previous experiments under saline conditions showed that Stockosorb K 410 had no effect on the pH value of soil solutions (Chen, Zommorodi et al. 2003). However, applying potassium polyacrylate (BF), sodium polyacrylate (JP), and polyacrylamide mixed with attapulgite sodium (WT) treatments, reduced pH values at soil moistures of 27.1 and 42.0 vol.%, but increased pH values at 14.3 and 85.6% (Bai, Zhang et al. 2010); that shows that there is an interaction between polymer type and soil moisture on soil pH.

1.2.5 Effect of polymers on soil moisture

A close relationship was found between the amount of hydrogel-type polymers applied and volumetric water content of three different soil types (Abedi-Koupai and Asadkazemi 2006; Abedi-Koupai, Sohrab et al. 2008) **(Table 1.3)**. However, no significant increase in volumetric water content compared to control between hydrogel types with the same amount of hydrogel application was found. Superab 200, TarawatA100, and PRA3005A did not increase the volumetric water content of three soil types (sandy loam, loam, and clay), although there were considerable differences in volumetric water content between these soil types without these polymers. Similarly, application of Superab A200, a hydrophilic polymer, to two types of soil at 0.2% and 0.6% W/W ratio led to an increase of water availability by 2.6% and 5.0% in loamy sand and 1.9% and 2.0% respectively in a sandy clay loam (Dorraji, Golchin et al. 2010).

However, some results showed positive effects of polymers applied to sandy soil; Carboxymethylcellulose (RF) and isopropyl acrymalide (BF) increased water absorption 4 and 5 fold, respectively (Andry, Yamamoto et al. 2009). Similarly, hydrophilic polymers (Superab A200) applied at rates of 4 or 6g kg^{-1} (soil) of light soil texture had positive effects in reducing irrigation rates (Abedi-Koupai and Asadkazemi 2006). There was a close correlation between concentrations of cross-linked polymers and water holding capacity in sandy soil (Green, Foster et al. 2004; Andry, Yamamoto et al. 2009).

Correspondingly, saline soil mixed with 0.6% Stockosorb K 410 significantly increased soil moisture (0.31 kg water kg^{-1} soil compared to control 0.22-0.31 kg water kg^{-1} soil) (Chen, Zommorodi et al. 2003). Similarly, permabsorb, a cross-linked polymer, led to increased water holding capacity in pot experiments when mixed with peat, vermiculite and perlite (Flannery and Busscher 1982); The same effect was observed for polymers introduced to sandy soil mixed with bark and peat moss (Letey, Clark et al. 1992).

Table 1.3 Effects of different hydrogels on volumetric water content (%) at water available content in different soil types (Abedi-Koupai and Asadkazemi 2006; Abedi-Koupai, Sohrab et al. 2008)

Soil types	**Hydrogel types**	**Amount of hydrogel addition (g kg^{-1})**				
		0	**2**	**4**	**6**	**8**
Sandy-loam	PR3005A	3.17	5.31	7.01	8.15	10.06
	TarawatA100	3.17	5.28	5.90	7.49	8.32
	Superab A200	4.54	-	9.84	10.33	-
Loam	PR3005A	4.27	5.39	5.04	9.11	9.55
	TarawatA100	-	5.53	5.90	7.49	8.32
Clay	PR3005A	4.65	5.55	6.77	8.05	8.39
	TarawatA100	4.65	4.78	5.23	7.91	8.29
	Superab A200	15.90	-	17.89	19.40	-

Note: Superab A200 (2006), PR3005A, TarawatA100 (2008).

Additional factors should be considered when polymers are applied to soil:

1) Soil moisture: The water absorption capacity of polymers sharply increased when the soil moisture content was close to the saturation point (Green, Foster et al. 2004)

2) Drying cycles: Increasing the number of drying cycles led to decreased water holding capacity of re-wetted hydrophilic polymers (Green, Foster et al. 2004)

3) The chemical composition of the polymer chain: when adding superabsorbent hydrogels (Stockosorb K 400, a highly cross-linked polyacrylamide with about 40% of the amide group hydrolysed to carboxylic groups) to the soil, soil moisture increased correspondingly with hydrogel levels (Hüttermann, Zommorodi et al. 1999).

Polymers can also affect saturated hydraulic conductivity (water movement through saturated media). This effect depends on the polymer type; i.e. saturated hydraulic conductivity of the isopropyl acrylamide treated soil increased significantly ($P < 0.05$) and linearly with increasing soil temperature, while that of sandy soil treated with carboxymethylcellulose showed a quadratic response (Andry, Yamamoto et al. 2009).

1.2.6 Effect of polymers on soil properties

Polymers were shown to affect soil bulk density, air-filled pore-space, nutrient content and water movement.

Greenhouse experiments showed that applying 0.2% of Polyacrylamide (PAM) within the top 0-7cm of silt-loam soils with a moisture content of 20% did significantly change soil/bulk-density (Steinberger 1990). Similarly, under field conditions with flood irrigation, PAM applied to clay loam soil at the rate of 650 kg ha^{-1} revealed effects on soil density (Terry and Nelson 1986). However, further research illustrated that Super- absorbent polymers (SAPs) could considerably reduce bulk-density, especially in moderate water deficit conditions; further, at rates of 0.05 to 0.3% SAP, bulk density decreased with increasing rates of SAP's (Bai, Zhang et al. 2010). Polymer effect depend on soil types i.e. when 6g Superab A200 kg^{-1} applied to soils Superab A200 improved air filled pore-space in sandy soils, but decreased air-filled pore-space in loamy soils (Abedi-Koupai and Asadkazemi 2006).

Polymer application to soil can also improve soil nutrient content. **Table 1.4** illustrates the effect of superabsorbent polymers on total nitrogen, available phosphate, and exchangeable potassium within 0-30cm soil depth. Soil nutrient increased with increasing amount of superabsorbent applied.

Under high salt content, the water absorption capacity of Stawet, Superhydro, and Hydrogel was reduced, resulting in a reduction of the soil swell index and an increase of water infiltration rate and water diffusivity (AI-Darby, Mustafa et al. 1990).

Table 1.4 Variations in total N, available P and exchangeable K contents with soil depths under different superabsorbent polymer treatments (Islam, Zeng et al. 2011)

Amount of Superabsor-bent(kg ha^{-1})	**Total N (g kg^{-1})**		**Available P (mg kg^{-1})**		**Exchangeable K (mg kg^{-1})**	
	0–0.15 m	**0.15–0.30 m**	**0–0.15 m**	**0.15–0.30 m**	**0–0.15 m**	**0.15–0.30 m**
0	1.01	0.95	22.1	17.1	127.7	119.2
10	1.09	0.93	21.7	18.8	132.8	124.9
20	1.20	1.09	26.7	22.4	141.6	122.5
30	1.37	1.19	32.1	24.2	148.4	132.4
40	1.36	1.30	34.3	26.1	151.9	136.1
Mean	1.21	1.09	27.4	21.7	140.5	127.0
LSD (0.05)	0.24	0.18	4.70	3.19	16.10	10.10

Note: LSD (0.05) – least significant difference at alpha equal 5%, irrigation was conducted once a week.

1.3 Hydrophilic polymers in interaction with plants

1.3.1 Plant growth responses to hydrophilic polymers

Table 1.5 summarizes the effects of polymers on plant growth or morphology of different species under drought conditions previously reported in literature. Almost all polymers improved plant growth under drought conditions. However, some hydrophilic polymers did not show any effects, or even negatively affected plant growth. Additionally, the effects of polymer application depended on the media they were applied to, the amount of polymers applied, the polymer types and plant species.

Positive effects of polymers on plant growth: Hydrophilic polymers significantly improved to radish shoot growth but caused a slight decline in tuber growth (Magalhaes, Wilcox et al. 1987). Dry biomass of *Populus euphratica* including leaf, stem and root growth significantly improved when grown in saline soil mixed with hydrogel (Stockosorb K410). Similar to Stockosorb K410, Agrisorb had a positive effect on the growth of cauliflower seedlings, including shoot and root growth, as well as leaf number, compared to the untreated control (Koudela, Hnilička et al. 2011). A superabsorbent polymer was reported to have positive effects on maize growth and grain quality parameters such as plant height, stem diameter, leaf area, and biomass accumulation as well as grain protein, sugar, and starch content (Islam, Zeng et al. 2011). In addition, the length and surface area of *Populus euphratica* roots in a hydrogel treatments was significantly increased compared to the untreated control (saline soil without hydrophilic polymer) (Chen, Zommorodi et al. 2003). A cross-linked type polyacrylamide revealed positive effects when applied to seedlings of *Lactuca sativa* L., *Raphanus sativus* L. and *Triticum aestivum* L. under water limited conditions as compared to conventional irrigation, thus reducing the drought risk for the crops (S'Johnson and Leah 1990). The survival rate of seedlings of *Pinus halepensis* in drying soil was twice as high when 0.4% Stockosorb K 400 was added. In addition, shoot and root vigor was approximately three times higher than the control grown under the same condition in pure soil (Hüttermann, Zommorodi et al. 1999). The presence of hydrogels, may stimulate the growth during periods of drought i.e. a hydrogel (prepared at laboratory scale by polymerisation of acrylamide (N,N-methylbis-acrylamide and mixed Na and K salts of acrylic acid) applied to soil at rates of 0.1%, 0.2% or 0.3% delayed reaching the wilting point in seedlings of barley (*Hordeum vulgare L.*), wheat (*Triticum aestivum L.*) and chickpea (*Cicer arietinum L.*) by 4 to 5 days by releasing stored water to the seedlings under drought conditions (Akhter, Mahmood et al.

2004). *P. popularis* under full irrigation (control) did not response to either 0.5% Stockosorb and Luquasorb applied to the soil; however, this species responded well to these hydrophilic polymers under drought conditions. For examples, root, leaf, stem, and total biomass of *P. popularis* on soil treated with either Stockosorb or Luquasorb were significantly higher than those on untreated soil. Furthermore, Luquasorb had stronger effects on total biomass as than Stockosorb when *P. popularis* was subjected to drought and saline condition simultaneously **(Table 1.5)**. The application of Geohumus in the fields significantly increased the biomass of sunflower, rape, maize, buckwheat, and cocksfoot (Trimborn, Heck et al. 2008).

Neutral or negative effects of polymers on plant growth: Plant height, shoot diameter, and length green of *Cupressus arizonica* were not affected when either 4g or 6 g of Superab A200 kg^{-1} of soil were applied to the fields under 66% evapotranspiration replacement as compared to control conditions (untreated with 100% evapotranspiration replacement). However, the addition of the polymer allowed reducing the irrigation frequency in light soils albeit not in heavy soils (Abedi-Koupai and Asadkazemi 2006). This result was consistent with the result from another experiment conducted by Lamont and O'Connell (1987) who applied up to 1 kg Terro-Sorb® kg m^{-3} mixture (a peat moss, sand and rice hull medium) and 0.5 kg Austra-sorb kg m^{-3} mixture (dolomite and lime contained 2 kg m^{-3} superfine superphosphate (9% P), 0.5 kg m^{-3} Micromax®) with no significant effect on shoot weight of Petunia and Marigold at wilting point **(Table 1.5)**. Similarly, 2.79 kg m^{-3} Planta Gel® also revealed no effect on shoot weight of crape myrtle after a drought stress cycle (Davies and Castro-Jmenez 1989). Maize shoot weight (Silberbush, Adar et al. 1993b) and pine branch (Hüttermann, Zommorodi et al. 1999) under dehydration were not improved when a low rate of Agrosoak (0.15%) and Stockosorb K400 (0.04%) were applied, respectively.

Similarly, the TerraSorb Hydrogel showed no significant improvement on either the delaying of the wilting point nor final shoot biomass of petunias (Lamont and O'connell 1987). In some cases negative effects of polymer application were reported. Higher doses of Perabsorb mixed with peat, vermiculite, and perlite (3.2 and 6.4mg L^{-1}), for example, did not improve plant vigor, but decreased the yield of *Azalea* and *Impatiens,* as increased water holding capacity lead to a strong reduction in oxygen supply to the roots (Flannery and Busscher 1982). Another result showed that although adding polymers to the soil helped improving soil water content, reducing the frequency of irrigation was not sustainable for the yield of crops. No significant differences in growth and germination were observed between polymer and control treatments, whereas overdosing of a cross-linked polymer (20x the recommended dose) reduced the yield of beans by 17% (Green, Foster et al. 2004).

In addition, different polymers applied to the same plant species under the same conditions can induce different results. According to results from previous experiments (Davies and Castro-Jmenez 1989) where starch and organic polymers were applied to crape myrtle, hydrogels not always positively effect crops. The starch co-polymer was comparably more effective for biomass development under drought conditions than the organic hydrogel, although both significantly increased biomass under well-watered conditions. Polymer effects on plants may not be additive. Combining several polymers may lead to conflicting effects on plants. Studies with a cross-link co-polymer agronomic gel (AGRO) showed positive effects when applied to three-month-old seedlings of *Citrumelo,* with regards to growth, water use efficiency, and nitrogen uptake (11-45% of control) when cultivated in sandy soils, whereas the acrylamide/acrylate co-polymer (PAM) did not induce beneficial effects. Incidentally, mixing PAM and AGRO showed the same results as when PAM was used alone (Syvertsen and Dunlop 2004). In contrast, (Rughoo and Govinden 1999) have shown for rainfed conditions that both organic soil amendment and hydrogel credibly displayed their usefulness in increasing the survival rate of tomato seedlings; however, although the organic soil amendment more strongly improved seedling survival rates the best results were achieved with a mix of the organic soil amendment and the hydrogel. Finally, the effect of hydrophilic polymers on plant growth may depend on the severity of the water deficit in the soil. For example, (Yasin and Rashid 2000) that with strongly reduced irrigation (1/3 field capacity) all four soil amendments applied (Terrasob, Aquasorb, Hydrogrow-400 at 1g kg^{-1} soil and farmyard manure at 10g kg^{-1} soil) resulted in significantly decreased sunflower height, leaf area and dry weight compared to control conditions (without soil conditioners and maintained at field capacity).

Table 1.5 Effects of polymers on morphology and productivity plants under drought conditions

Commercial name	**Name or formula of chemicals**	**Amount applied**	**Effect-iveness (0/+/-)**	**Plant**		**Productivity/ morphology**	**sources**
				Commen name	**Scientific name**		
Perabsorb		3.2 and 6.4 mg L^{-1}	-/-	Azalea Impatients	-	shoot	(Flannery and Busscher 1982)
Celanese		1 and 5%	-/-	Zinnia	*Zinnia elegans*	Height	(Furuta and Autio 1988)
Agrosoke		1 and 5%	+/-	Zinnia	*Zinnia elegans*	Height	(Furuta and Autio 1988)
Terrasorb		1 and 5%	+/+	Zinnia	*Zinnia elegans*	Height	(Furuta and Autio 1988)
IGETA-GREEN P	a vinyl alcohol-acrylic acid copolymer sodium salt	0.2%	+	Radish	*Raphanus sativus L.*	Shoot	(Magalhaes, Wilcox et al. 1987)
Terro-Sorb®		0.25, 0.5, 1 kg m^{-3}	0/0/0	Petunia		Shoot weight	(Lamont and O'connell 1987)
Austra-sorb		0.5 kg m^{-3}	0	Marigold		Shoot weight	Lamont and O'connell 1987)
Terro-Sorb®		1.47kg m^{-3}	+	Crape myrtle	*Lagerstroemia indica*	Shoot weight	(Davies and Castro-Jmenez 1989)
Planta Gel®		2.97kg m^{-3}	0	Crape myrtle	*Lagerstroemia indica*	Shoot weight	(Davies and Castro-Jmenez 1989)
	cross-link polyarcilamide	0.5, 1, 2, 5 g kg^{-1}	+/+/+/+	Lettuces	*Lactuca sativa L, Raphanus sativus L, Triticum aestivum L*	Shoot	(S'Johnson and Leah 1990)
	-polyacrylamide -starch co-polymer -polyvinylalcohol	0.1, 0.2, 0.5%	+/+/+	Bar ley Lettuce	*H. vulgare cv. 'Tasman'* *L. sativa cv. 'Webb's Wonderful'*	Shoot weight	(Woodhouse and Johnson 1991)
Agrosoak®	Polyacrylamide	0.15, 0.3, .45%	0/+/+	Maize	Zea mays *L.*	Shoot weight	(Silberbush, Adar et al. 1993b)
Stockosorb K 400	cross-link polyarcilamide	0.04,0.08,0.12, 0.20, 0.40%	0/+/+/+/+/+	Pine	*Pinus halepensis*	More branched and adventitious	(Hüttermann, Zommorodi et al. 1999)

Note: 0, -, + indicate neutral, negative and positive effect of polymers corresponding to amount applied in column, respectively.

Table 1.5 Effects of polymers on morphology and productivity plants under drought conditions (continued)

Commercial name	Name or formula of chemicals	Amount applied	Effect-iveness (0/+/-)	Plant: Commen name	Plant: Scientific name	Productivity/ morphology	sources
Stockosorb K410		0.6%	+	Poplar	*Populus euphratica*	Leaf, stem, root	(Chen, Zommorodi et al. 2003)
	cross-link polyarcilamide	448kg ha^{-1}	-	Beans (Othello, Bill Z.)		Germination Biomass	(Green, Foster et al. 2004)
Superab A200		4, 6 g kg^{-1}	0/0	Ornamental plant	*Cupressus arizonica*	Height, shoot diameter	(Abedi-Koupai and Asadkazemi 2006)
Geohumus®		2.5g kg^{-1}	+	Sunflower Oilseed rape		Biomass	(Trimborn, Heck et al. 2008)
Geohumus®		5tones ha^{-1}	+	Buck wheat, Cocksfoot, Sunflower		Biomass	(Trimborn, Heck et al. 2008)
Stockosorb500LX	cross-linked poly potassium-co-(acrylic resin polymer)-co-polyacrylamide hydrogel	0.5%	+		*Populus popularis*	Root, leaf, stem, plant	(Shi, Li et al. 2010)
Luquasorb	potassium polyacrylate	0.5%	+		*Populus popularis*	Root, leaf, stem, plant	(Shi, Li et al. 2010)
Agrisorb		3g L^{-1}	+	Cauliflower	*Brassica oleracea*	Shoot, root, number of leaves	(Koudela, Hnilička et al. 2011)
superabsorbent		10, 20, 30, 40 kg ha^{-1}	+/+/+/+	Corn	Zea mays *L.*	Leaf area, biomass	(Islam, Zeng et al. 2011)

Note: 0, -, + indicates not, negative and positive effect of polymers corresponding to amount applied in column

1.3.2 Leaf and xylem ABA and xylem pH

Drought resulted in plant biochemical changes (Bohnert, Nelson et al. 1995); for instance, an increase in plant xylem pH (Bahrun, Jensen et al. 2002; Wilkinson and Davies 2002; Schachtman and Goodger 2008) as well as increases in leaf and xylem ABA (Zhang and Davies 1989; Christmann, Weiler et al. 2007; Asch, Bahrun et al. 2009). The increase in xylem pH increase in combination with increased xylem ABA (Asch, Bahrun et al. 2009) causes reductions in stomatal conductance (Thompson and Mulholland 2007). Consequently, plant growth rates decrease (Khan, Hussain et al. 2001). Up to now, however, little is known about effects of hydrophilic polymers added to the soil on plant biochemical traits or processes. Except, a previous experiment showed that under shorter dehydrated condition, superabsorbent polymer application could release water for plant leading to plant to stress condition later; however, longer stress took place, plant would be put in stress trouble after using up water stored from polymer. That reasons why ABA concentration of maize increased as grown on soil mixed with superabsorbent (Moslemi, Habibi et al. 2011). So investigating further effect of hydrogel application on plant biochemical traits under drought is essential.

1.3.3 Plant water status and leaf gas exchange

In general, plant leaf water potential decreases with progressive soil drying (Harris and Health 1981; Sanchez, Hall et al. 1983; Bahrun, Jensen et al. 2002) leading to stomatal closure (Harris and Health 1981). In the following paragraphs the effects applying hydrogel to drying soils on leaf and shoot water potentials as well as stomatal conductance and transpiration will be reviewed.

Water content and water potential (Ψ_w) have been widely used to quantify the water deficits in leaf tissues. Leaf water content is a useful indicator of plant water balance, since it expresses the relative amount of water present in the plant tissues (Yamasaki and Dillenburg 1999). According to results from previous experiments in an arid and semi-arid region of Northern China, superabsorbent application improved the relative water content in maize leaves at 4, 6 and 8 weeks after sowing (Islam, Zeng et al. 2011). This parameter showed a close correlation with the amount of superabsorbent applied.

Another result illustrated the relationship between the water potential of *Pinus halepensis* seedlings and the applied amount of Stockosorb K400 under drought stress. Compared to the control, Stockosorb K400 prolonged the maintenance of a high water potential of *Pinus halepensis* seedlings (Hüttermann, Zommorodi et al. 1999). Equally, evapotranspiration of *Pinus halepensis* seedlings increased with increasing amount of Stockosorb K400 (0.04-0.4%) mixed with soil during four weeks of drought (Hüttermann, Zommorodi et al. 1999). Several studies analyzed the effect of polymers on leaf gas exchange parameters such as stomatal conductance, transpiration, and CO_2 uptake. In plants under water deficit, stomatal conductance was reduced (Sanchez, Hall et al. 1983; Vitale, Tommasi et al. 2009) and it has been shown that hydrophilic polymers can improve soil moisture content (Chen, Zommorodi et al. 2003; Green, Foster et al. 2004; Andry, Yamamoto et al. 2009). Thus, polymer application is expected to improve plant stomatal conductance under drought conditions, which was confirmed by Shi, Li et al. (2010) when applying Stockosorb and Luquasorb polymers (applied at 0.5%) to *P. popularis* under water shortage and finding increased stomatal conductance values as compared to the control.

Drought severity not only affects the plant's water status but also the effectiveness of polymers. This was demonstrated by Davies and Castro-Tmenez (1989) in a study on crape myrtle. Under severe drought (20% weight loss of container), two polymers namely Viterra Planta-Gel and Terra-Sorb had no effect on leaf water potential of crape myrtle as compared to plants with no polymer applied. However, under less severe drought conditions (10% weight loss of container) polymer application significantly improved leaf water potential and transpiration rate

Similarly, Stockosorb and Luquasorb polymers also increased transpiration in *P. popularis* under drought conditions or water deficit combined with saline conditions *(Shi, Li et al. 2010)*. Application of polymers to sandy soils, mixed with bark and peat moss increased water availability for Marigold. However, the polymers did not contribute to water conservation as there was no difference in evapotranspiration of Marigold between control and polymer treatments (Letey, Clark et al. 1992).

1.3.4 Hydrophilic polymer effects on plant root-shoot partitioning

Root to shoot ratios differed according to the polymer type, rate, and depth of application. For example, an experiment was conducted by Ei-Amir, Helalia et al. (1991) on maize including three treatments related to the mode of application of Acryhope and Aquastore (at 0.5 or 1% mixed at top half pot, whole pot and bottom half pot), irrigation was established when soil moisture was reduced to 60%. With Acryhope polymer at 0.5% top half treatment, which has a low water holding capacity, the root shoot ratio is much higher than whole depth, bottom half treatment at the same amount (0.5%) and at double amount (1%) with three application methods but was lower than control (without polymer) while Aquastore polymer at both 0.5 and 1.0% with three application methods were considerably lower than that on control and Acryhope at 0.5% (EI-Amir, Helalia et al. 1991)**.** Another experiment illustrated that a hydrogel (linear acrylate copolymer) at 27 and 55 mg L^{-1} had no difference in Citrumelo root shoot ratio (Syvertsen and Dunlop 2004). This is completely consistent with result of Davies and Castro-Jimenez (1989) where organic hydrogel (at 2.97kg m^{-3}) or Terr-sorb® (1.47 kg m^{-3}) application had no effect of *Lagerstroemia indica* shoot and root ratio under both stress (20% weight loss of container capacity) and non-stress drought condition (10% weight loss of container capacity).

1.3.5 Hydrophilic polymer effects on water use efficiency (WUE)

Polymer application showed positive effect on plant WUE that depends on type, dose of polymers, applied media (soil type and drought level) and method (Woodhouse and Johnson 1991), e.g. the result illustrated the effects of polymers in variable doses on WUE of barley and lettuce i.e. in comparison; there were different responses to polymer types and treatment level in WUE of barley and lettuce. Lettuce WUE increased with increasing treatment level of polyacrylamide while applying 0.1% polyacrylamide to barley showed highest WUE. However, application of 0.1% polyvinylalcohol treatment resulted in highest WUE of barley, while polyacrylamide led to highest WUE at 0.5%. Similarly, applying at 0.5% Acryhope WUE of corn was superior to Aquastore at the same amount and double amount of Acryhope (at 1.0%) resulted in decrease of corn WUE (EI-Amir, Helalia et al. 1991).

Response to polymers depends on plant types and soil moisture condition. In the case of Geohumus application, sunflower and rape grown into soil with Geohumus under drought condition showed significantly higher WUE than control while maize WUE did not respond to Geohumus under both wet and drought condition. Additionally, under wet condition, Geohumus application showed considerable increase of rape WUE but sunflower (Trimborn, Heck et al. 2008).

Treatment effects depended on media in which the polymer was applied, i.e. application of Superab A200 at the rate of 0.6% in loamy sand soil or at the rate of 0.2% in sandy clay loam soil resulted in the highest corn WUE (Dorraji, Golchin et al. 2010). However, under 1/3 field capacity, soil conditioner treatments including Terrasob, Aquasorb, Hydrogrow-400 at 1g kg^{-1} soil and farmyard manure at 10g kg^{-1} showed significant decrease in WUE compared to the treatment under field capacity without soil conditioner (Yasin and Rashid 2000). A cross-linked type polyacrylamide (ALCOSORB®400) allows not only the increase of water holding capacity of sandy soils, but also the mitigation of water loss from vacuoles leading to both the reduction of irrigation frequency and rates. As a consequence, ALCOSORB®400 contributed to increasing water use efficiency of soybean (Sivapalan 2006).

Additionally, plant also responded to application method of polymer illustrated that Acryhope at 0.5% applied only to the top half pot, resulted in higher corn WUE compared to the bottom half and whole depth as well (EI-Amir, Helalia et al. 1991).

2 MATERIALS AND METHODS

In total 3 different experiments were carried out in the greenhouse and the laboratory of the University of Hohenheim, Stuttgart, Germany from 2009 - 2011 to analyze Geohumus under different environmental conditions, its long-term performance, and effects on plant growth and drought responses. A first experiment was designed to analyze the WHC and restorability of Geohumus. In detail, the performance of Geohumus was tested under different temperatures, soaking time, various sources of solution, dose of nutrient solution, selected salts (salt content and types of valance, incorporation depth, and its restorability as well.

The second experiment called ‘drought spell experiment, main objective is morphological and physiological responses of two maize cultivars under prolonged water deficit as influenced by Geohumus application such as Geohumus effects on changes of soil moisture, growth, non-hydraulic signals (ABA]$_{leaf}$ and [ABA]$_{xylem}$, and pH$_{xylem}$), and plant water status and leaf gas exchange (stomatal conductance (Gs), transpiration (E) and photosynthesis (A)) of two maize cultivars under simulated drought spell and full irrigation condition. Correspondingly, Geohumus effects on root-shoot partitioning, assimilate remobilization, water use efficiency were investigated. The third experiment, a split root system, with main objective is to estimate effects of Geohumus and two soil types (sandy soil and compost) on drought induced root-shoot communication of two maize cultivars through non-hydraulic signals (ABA]$_{leaf}$ and [ABA]$_{xylem}$, and pH$_{xylem}$), plant water status (leaf and root water potential and leaf and xylem osmotic potential) and Gs.

2.1 Impact of selected abiotic factors on Geohumus WHC and restorability

2.1.1 Determination of Geohumus water holding capacity

Teabags were used to determine the WHC in mL water g^{-1} of Geohumus as previously described by (Andry, Yamamoto et al. 2009) and referred to as the ‘teabag method’. In this method, commercial teabags (2.5 x10 cm) were used to estimate the WHC of Geohumus (**Fig. 2.1**). One gram of Geohumus was placed into a teabag and immersed in 100 ml of a prepared medium (Andry, Yamamoto et al. 2009) for two hours (Chen and

Zhao 2000). The teabags were taken out of solution and suspended on the solution-containers sides overnight. The "hydrated" Geohumus was taken out of the teabags and blotted on tissue paper to decant excess water and weighed on a fine electronic balance (*Precisa Gravimetrics AG Dietikon, Switzerland)*. Finally, the WHC of Geohumus was calculated using the formula below:

WHC (mL g^{-1}) = $(W_2 – W_1)/W_1$ (equation 1) (Chen and Zhao 2000)

Where: W_2 and W_1 are the weight of the swollen and fresh (un-swollen) Geohumus respectively.

Fig. 2.1 Tea bag used for Geohumus water holding capacity

2.1.2 Temperature

To estimate temperature effects on the WHC of Geohumus, 1g Geohumus was placed into each teabag and immersed into distilled water (control) and maize nutrient solution **(Table 2.3)**. Three replicates were incubated at controlled temperatures (0, 10, 20, 30, 40, and 50ºC) in a Growth chamber (Percival Scientific, Germany) for 2 hours. The WHC capacity was determined with the "tea bag" method described in section 2.1.1.

2.1.3 Immersion duration

To study the effects of incubation time on WHC, 1g of Geohumus was incubated with 3 replicates in distilled water and nutrient solution **(Table 2.3)** for 2, 4, 6, 10, 20, 30, 40, 50, and 60 hours. After incubation the WHC of Geohumus was determined (see 2.1.1) at 25°C.

2.1.4 Salts (various sources of solutions, dose of nutrient solution and selected salt concentration and types of valence)

To estimate effect of salts comprising salt types, salt concentration, and valence type on WHC of Geohumus, three separate trials were conducted in the laboratory, the applied methods were explained in detail following:

a) Variable sources of solutions

A hypothesis was raised that different solutions leading to difference in ‘quality’ of solutions; that would differently cause Geohumus WHC. To check the effect of different solutions on WHC of Geohumus, there were 6 media used for this trial shown in detail in **Table 2.1.** Soil extracts were obtained in a 1:1 distilled water, soil or compost (W/W) ratio as described by (Freeland, Richardson et al. 1999). Samples were left for 30 minutes to stabilize. The supernatant was then filtered through a paper. pH and EC were measured using a pH meter (pH526, WTW, Germany) and EC meter (Cole-Parmer instrument Co., Chicago, USA).

Table 2.1 pH and EC value of media used to imbed Geohumus

Media	**EC(µmhos cm^{-1})**	**pH**
Distilled water	18	6.4
Tap water	33.5	7.45
Soil (1:1 w/w)	180	7.24
Soil (1:1 w/w) + 100% nutrient solution (*)	6610	7.16
Compost (1:1 w/w)	1746	7.32
100% nutrient solution (*)	5000	7.11

Note: (*) section 2.1

100ml of solutions were used as soluble media into which teabags containing 1 g of Geohumus were immersed for 2 hours to estimate WHC of fresh Geohumus (3 replicates).

b) Dose of nutrient solution

To understand the effect of nutrient concentrations on the WHC of Geohumus, 2 g of Geohumus were placed in plastic containers with meshed bottoms, and immersed in nutrient solution (**Table 2.3**) treatments for 2 hours. The nutrient solutions were prepared in concentrations reflecting 0 (control), 25, 50, 75, 100, 200, 300 and 400% of the physiological demand of maize during the life cycle; this nutrient solution was recommended by Bonn University and checked for exploratory experiment at Hohenheim University and made for sandy soil (Cottbus, Germany) used for effects of Geohumus on plant performance in this research. Aluminum foil was used to cover the containers' tops, in order to prevent evaporation. Excess nutrient solution was decanted, and interstitial water was held between gel particles; WHC was determined when the weight of swollen Geohumus was stable and calculated by formula (1).

c) Salt concentration and types of valance

The WHC of Geohumus was not only affected by the properties of salt solutions, but also by the electrical conductivity of these solutions as discussed in section ***1.2.2.*** Additionally, results from sources of solutions and dose of nutrient solution sections could not explain which specific salts or elements affect on Geohumus WHC**.** In this section, we carried out two separate trials comprising effects of relative salt content and type of salts on WHC of Geohumus in turn.

* **Relative salt content:** Some salts were chosen to estimate their content relatively affecting on water absorption of Geohumus. 1g of Geohumus was placed into a teabag, each replicated 3 times for each salt and concentration. The teabags were submersed in solutions of KCl, NaCl, K_2SO_4, $FeSO_4.7H_2O$, $MgSO_4.7H_2O$, KNO_3, NH_4NO_3, $Al_2O_{12}S_{31}$ and in mixtures (NaCl, KCl, K_2SO_4, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$) with various concentrations (0.2, 0.15, 0.125, 0.1, 0.05, 0.04, 0.03, 0.02, and 0.01M) for 2 hour under laboratory condition (temperature about 25^{o}C).

* **Types of valance**: To estimate the relative effect of ions on WHC of Geohumus, the fact of EC was eliminated through the addition of de-ionised water, thereby equalizing the EC values between of specific salt solutions. Group with mono-valance (K_2SO_4, KCl, NaCl) and multivalancy ($MgSO_4$ and $FeSO_4$) salts were chosen and diluted according to various concentration 0.2, 0.15, 0.125, 0.1, 0.05, 0.04, 0.03, 0.02, and 0.01M; then EC values of these solution were determined by EC meter (Cole-Parmer instrument Co., Chicago, USA); EC values were finally converted to salt concentrations (g L^{-1}) by the following formula:

TDS (mg L^{-1}) = EC (mmhos cm^{-1}) x 640

Where TDS: total dissolved solid, 640: conversion coefficient

2.1.5 Impact of incorporation depth

To analyze incorporation depth on WHC of Geohumus, a pot experiment was conducted. Plastic Pots used in this experimented were composed of four plastic rings which were 12.5 cm in height and 15 cm in diameter, resulting in a single column of 50cm in height, with a total volume of 8831.25cm^3. The plastic rings were held in place using "pressure sensitive adhesive tape" (cellotape). There were two treatments for this trial: (1) control (14kg sandy soil per pot) and (2) nutrient treatment (14kg sandy soil per pot sandy soil mixed with nutrient solution for maize). In the nutrient treatment, soil was mixed with 100% nutrient stock solution and left to air dry for 3 hours before filled into pot. The pots were filled layer by layer, according to the depth of the individual plastic rings (12.5cm). 3 teabags filled with 2 g of Geohumus were separately placed between each layer of soil (32.5 45; 20-32.5; 7.5-20 and 0-7.5 cm). An Irrigation system with 19 adjustable tips to control the water flow (total 15ml water per minute) was used to irrigate the soil from the top. Irrigation was stopped when water was observed flowing from the bottom of the pots. The saturated pots were covered with aluminum foil to prevent evaporation. Sampling occurred when water stopped flowing from pots. (1) Soil samples were taken at 3 positions for the measurement of EC and pH, using the cylinder tool 'Soil gauge' with a diameter of 2.7cm; resulting in 12 soil samples per pot. Soil samples were air-dried (in the greenhouse), milled and passed through a 2mm mesh size sieve before measuring pH and EC following the procedure described section ***2.1.4*** (2) The teabags were taken out of the soil and preserved in aluminum boxes to prevent evaporation. The swollen Geohumus was removed from the teabags and weighed, in order determine WHC.

2.1.6 Used Geohumus restorability

As discussed in section 1.2.2, polymer WHC was affected by salt concentration and types and according to Geohumus Company, Geohumus can maintain WHC up to 15 times its own weight in water. However, in salt solution or soil media were not mentioned. Whether Geohumus WHC mediated by salt solution or soil condition is questionable. Two different trials were conducted following.

a) Used Geohumus (collected from soil)

Two sources of used Geohumus collected from two types of separate experiments were used to estimate Geohumus recovery.

* **Geohumus source from drought spell experiment**: the experimental design such as pots, soil filling, nutrient application, seedling, saturating and irrigation was the same like **Section 2.2.2**. However, Geohumus in this experiment was applied to whole soil volume of the pot instant of second layer of pot. Geohumus was separately collected from 4 layers of pots.

* Geohumus source from Split root system (SRS): this source was directly collected from experiments in **Section 3.3**. Geohumus on the same cultivation media (sandy soil or compost) under three water regimes (FI_{SRS}, PRD and DI) collected from was mixed together.

Geohumus collection was established when root washing process was carried (**Section 2.2.4)**. Geohumus samples collected were air-dried then subsequently immersed distilled water for 2 and 24 hours to determine WHC by tea bag method.

b) used Geohumus (from different salt solutions)

To measure whether it is possible for Geohumus to recover its maximum WHC in distilled water after having been immersed in saline solutions, 1g of Geohumus was immersed in the following salt solutions for 2 hours namely:

- $FeSO_4.7H_2O$, $Al_2O_{12}S_{31}$ (strongest effect group);
- KNO_3, and

– NH_4NO_3 (lowest effect group)

The teabags were removed from the different salt solutions and rinsed in 100ml H_2O for 10 minutes. The teabags were subsequently submersed in distilled water for a period of 2 hours, after which WHC was calculated as described above.

2.2 Morphological and physiological responses of two maize cultivars under prolonged water deficit as influenced by Geohumus application

2.2.1 Environmental data

TinyTag data logger (TGP-4500, Gemini data logger, Chichester, UK) was installed about 2cm from tip of leaf to record climate condition (daily mean temperature and humidity) during period of experiments in the greenhouse.

Experiment 2 was carried out on two maize cultivars (Companero and Mikado) over March 4th to May 5th 2010.

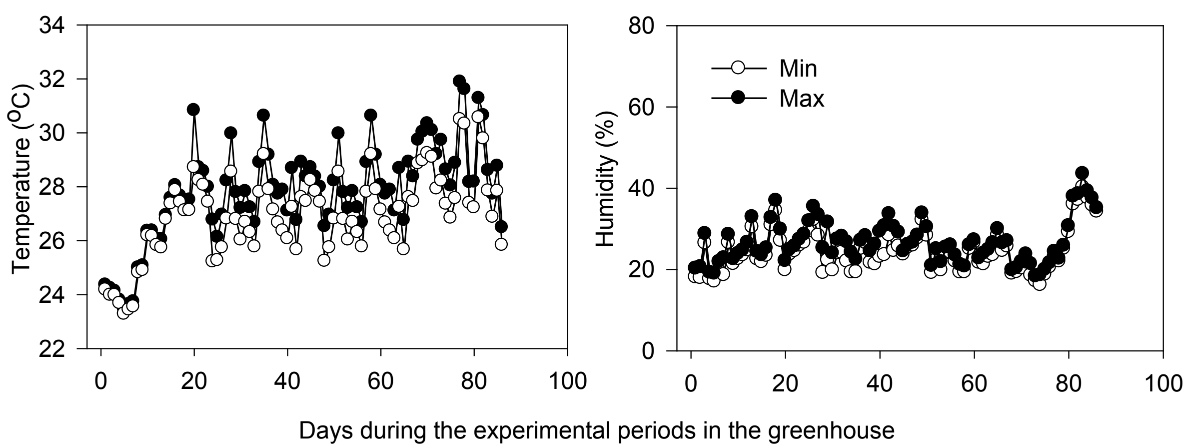


Fig. 2.2 The kinetics of temperature and humidity for the duration of the experiments

The mean minimum and maximum temperatures over the course of experiments were about 20.92-23.36 and 37.43-38.91°C, correspondingly; mean minimum and maximum humidity ranged from 8.78 to 10.93 and from 41.47% to 50.40, respectively (**Fig. 2.2**).

2.2.2 Experimental design

Greenhouse pot experiments were carried out to analyze how Geohumus in combination with different soil water contents affect the physiology, morphology and productivity of two maize cultivars. Experiment 2 was conducted in drought spell model with difference cultivar (Mikado or Companero).

* **Cultivars**: two cultivars were chosen for this research based on their morphology. Mikado, an energy maize prototype, shows vigorously growth and great leaf area while Companero, silage and grain maize, possesses medium leaf area and growth. Greater leaf area leads higher water demand (Schittenhelm, 2012) and small leaf area result in drought tolerance (Mickelbart 2010). It is hypothesized that difference in leaf area between Mikado and Companero may lead difference in drought response.

Fig. 2.3 Pots used for drought spell experiment in the greenhouse

* **Nutrient preparation:** The application of nutrients was based on the nutrient demand of maize (expected nutrient content in soil = measured nutrient content in soil plus additional fertilizer). As Geohumus contains only small amounts of nutrients, such as nitrogen and potassium, the amount of nitrogen and potassium was adjusted to ensure equal nutrient supply between control and the Geohumus treatments. e.g., the control treatments received 1.801g NH_4NO_3 and 2.624 K_2SO_4, while Geohumus treatments

received 1.790 and 2.607g respectively (**Table 2.2**). The used salts were dissolved in de-ionized water to produce ‘stock nutrient solutions’.

Table 2.2 Nutrient solution used for a plant on drought spell experiments

Sort of Salts	Amount of salt (g)	
	Control	Geohumus treatment
NH_4NO_3	1.801	1.790
$NaH_2PO_4*H_2O$	4.961	4.961
K_2SO_4	2.624	2.607
$MgSO_4*7H_2O$	4.260	4.260
$C_{10}H_{12}Fe_2NaO_8$	0.161	0.161
$ZnSO_4\ 7H_2O$	0.022	0.022
$MnSO_4H_2O$	0.021	0.021
$CuSO_4XH_2O$	0.125	0.125
H_3BO_3	0.008	0.008

* **Cultivation:** The pots used for drought spell experiments were constructed from PVC tubes with 0.15m internal diameter, capacities of 9 L, and 0.5m high and combined by tape from four equal compartments of 0.125m (**Fig. 2.3**). Each cultivar consisted of 64 pots (52 pots with plant and 12 without plant for evaporation measurement), 2 Geohumus levels (40g pot^{-1} and 0 g pot^{-1}(control)), 2 water treatments (control: Full irrigation and treatment: drought spell), 3 replicates, 5 harvest times. Air-dried and sieved (2mm) sandy soil from Cottbus, Germany was used for the experiments classified as ‘quaternary sand’ (Gerwin, Schillem et al. 2011). Filling progress was carried out step by step; 2kg of sandy soil were mixed with stock nutrient solution and allowed to air dry; then well-mixed with 12 kg sandy soil. The control treatment was filled with 14 kg sandy soil per pot; for the Geohumus treatment 40 g Geohumus was mixed into the soil of the pot's second compartment. All pots were saturated by irrigation system with 15 ml water per minute from tops. To obtain homogeneous population, two germinated seeds were transferred to a pot when seeds sprouted buds and roots (26 pot for control and 26 pots for Geohumus treatment); one plant per pot was chosen when seedling was about 3 cm in height. High

pressure sodium lamps (PL SON-K-400, DHlicht GmbH, Wülfrath, Germany) were used to supply light for maize during experiments with 12 hours per day from 6 am to 18 pm.

* **Watering and onset of drought treatment**: water source was used for during experiment is water tap. From germination to 30 age days, maize was daily watered to ensure soil moisture about 90% of field capacity (soil moisture was controlled daily; big balance (PCE-HPS60, capacity: 60kg ± 1g) was used to maintain field capacity by replenishing water loss from evapotranspiration). At 30 days of age, drought spell was stimulated; two water regimes were applied, including (1) full irrigation (FI, 100% field capacity) and (2) progressive drought (slow drying-out close to permanent wilting point) or drought spell (DS).

* **Samplings:** samplings were conducted according to relative soil moisture status (**Fig. 2.3)** to determine gas exchange, physiology, and morphology (leaf area) and productivity (leaf, shoot, root, stem weight) of plants, as well as at all sampling days described in detail below.

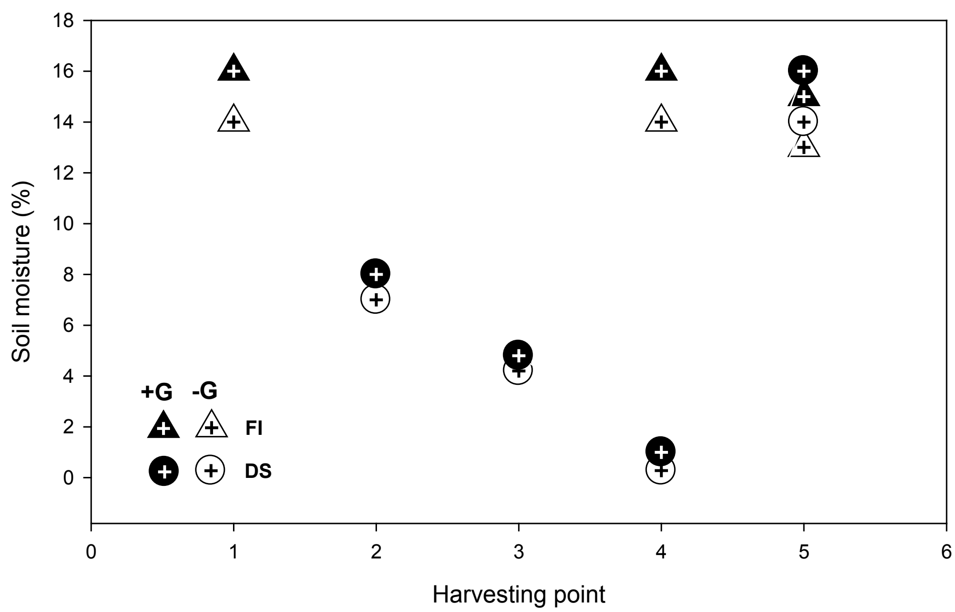


Fig. 2.4 Planning diagram for harvesting of a typical drought spell experiment

Note: +G: sandy soil plus Geohumus (Geohumus treatment); -G: sandy soil (control); FI: full irrigation; and DS: Drought spell.

The **Fig. 2.4** is diagram that explains how expected soil moistures on control and Geohumus treatment change under drought spell (DS) and full irrigation as well. At onset (harvesting point 1), when drought spell simulated, soil moisture on Geohumus would be higher than that on control and regularly decrease to lowest point (harvesting point 4) before increasing again when re-watering would be carried out at harvesting point 5 (drought spell ended). There were 3 and 5 harvesting points for FI and DS respectively. Each symbol (circle or triangle) indicates for three pots equal three replications; so in total 32 pots with plants per an experiment would be harvested.

2.2.3 Soil analysis

*** Soil moisture content (SM)**

A ‘Soil gauge’ (2.7cm diameter) was used to take soil samples. Soil samples were taken from the pot experiment at depths of 0-7.5cm, 7.5-20cm, 20-32.5cm and 32.5-45cm, after root water potential had been measured. Soil water content was measured after drying at 105º for 24h. Water content was calculated as follows:

$$SMC\ (\%) = (W_2 - W_3) \times 100 / (W_3 - W_1)\ \text{(Craze 1990)}\ (2)$$

Where: W_1: weight of empty container; W_2: total weight of weight of empty container plus wet soil mixed with Geohumus; and W_3 total weight of weight of empty container plus wet soil mixed with Geohumus.

*** Soil bulk density (SBD)**

To obtain this parameter, we only made of use data of mass dry soil and bulk volume from section soil moisture and calculated by following formula:

SBD = mass dry soil (g)/ bulk volume soil (cm^3) (Hanks and Ashcroft 1986)

*** Soil matrix potential – pF curves**

Sandy soil collected from Cottbus, Germany was air-dried, ground and sieved (2mm). Three replicates of 160g dried soil and 160g dried soil mixed with 0.41g Geohumus were filled into a cylinder (100cm^3) Rate of Geohumus and sandy soil on this trial was exactly equal with that on drought spell experiment (40g Geohumus per 14 kg sandy soil). This trial was conducted at the laboratory, Institute of Soil Science and Land Evaluation, Hohenheim University with sandbox method including three set for pF determination;

Sandbox for pF determination wet range (pF 0-2); Sand/kaolin box for pF 2-2.7; and membrane apparatus for pF 3-4.2.

2.2.4 Growth

*** Leaf area**

The MK2 Area Meter (Delta-T Devices Ltd., UK) was used to measure green leaf area (LA_1). Wilting leaves were embedded in water until they had recovered before scanning. Determination of Leaf Area of leaves sampled for the determination of leaf water potential and ABA concentration was conducted by measuring the length and maximum width of leaves, and applying the following formula:

LA_2 = length x maximum width x 0.75 (Turner 1975)

Total leaf areas involved LA_1 plus LA_2.

*** Root washing:** root samples were separately washed from the pot experiment at depths of 0-7.5cm, 7.5-20cm, 20-32.5cm and 32.5-45cm step by step; at first, soil with root was immersed in water until soil became soft; next step was to separate root from soil by sieve (1mm) roughly; using tap to segregate roots and soil or unwanted objects finally.

* **Root length analysis**: some special devices were used to determine root length data including a box with three lamps inside allowing light coming out from top only; a container made from transparent material (Mica). Washed roots were spread into transparent container with clean water inside; a camera was fixed above the transparent container to take pictures. Root length data was analyzed from pictures by WinRhizo 2000.

*** Dry Matter (DM):** After harvest, the dry weight of leaves, stems, and roots was determined after drying at 80ºC for 48 h (Zhang and Davies 1989).

* The Dry Matter Partitioning Coefficient was determined to clarify the pattern of leaf, root and stem growth as affected by different irrigation regimes (full irrigation and drought spell). The Dry matter partitioning coefficient was calculated on the basis of changes in dry matter ($\Delta dw\ \Delta dw^{-1}$) between two sampling days.

2.2.5 Plant analysis

*** Xylem sap collection**

Sap collection was followed up root water potential determination explained in detail in 2.2.6 section. Sap collection was applied up to a maximum pressure of about root water potential (bar) + 20%; sap was collected for a period of about 15 minutes. The samples were preserved at -20^{o}C, until analysis.

*** Leaf and xylem ABA analysis**

Leaf samples for ABA analysis (mentioned in section 2.2.6) were freeze dried and subsequently ground in a ball tripulator (Hersteller). Both ABA concentrations of leaves and xylem sap were analyzed using an indirect Enzyme Linked Immuno-sorbent Assay (ELISA) procedure (Asch 2000).

2.2.6 Plant Water status

*** Leaf, root water potential**

Fully developed leaves were chosen to determine leaf water potential in a scholander pressure chamber after cutting the leaf from the stem at the collar. The selected leaf was covered by aluminum foil to prevent water loss, and was subsequently placed in the chamber. The pressure was increased until a drop of water was exuded at the tip of the main leaf vein, which extended out of the scholander pressure chamber; Leaf water potential was measured in bar. Finally, the leaves were divided into two samples for the measurement of osmotic potential and ABA concentrations. The samples were preserved at -20^{o}C, until analysis. Root water potential was measured in a similar way to leaf water potential, using a larger pressure chamber, after cutting the stem about 20cm above ground; after the determination of root water potential, the pressure was continuously increased in order to collect xylem exudates from the top of the stem, which extended out of the chamber. The results of both leaf and root water potential (bar) were converted to the unit MPa.

*** Leaf osmotic potential, and xylem osmotic potential**

Leaf samples from the greenhouse experiment were submersed in liquid nitrogen, in order to stop cell metabolism, allowing for the accurate measurement of physiological characteristics at point of sampling. Leaf and sap samples were stored at -20^{o}C until analysis.

The leaf samples taken out from the fridge; then leaf extract was collected by squeezing. The extract was centrifuged by Biofuge (fresco, made in Germany 1997) with 12.000 rounds per minute to produce a clear supernatant; pipetting 15µ supernatant and dreading by OSMOMAT 030-D (made in Germany 2009). Unit of value in osmol kg^{-1} was converted to MPa (1 osmol kg^{-1} = 2.479 MPa).

Similar to leaf samples, sap samples also taken out from the fridge and contained in the container that can prevent the light from outside until sap samples defrost completely. Sap sample was treated and determined xylem osmotic potential like leaf osmotic potential

2.2.7 *Gas exchanges*

Photosynthetic rate (A), transpiration rate (E) and stomatal conductance (Gs) of maize leaves were measured using a portable gas exchange fluorescence meter (GFS-3000). The center of the youngest fully developed leaf was chosen for the determination of these parameters. Measurements were taken in the morning, between 8 and 12am.

2.3 Effects of Geohumus and two soil types (sandy soil and compost) on drought induced maize root-shoot communication

To investigate root-shoot communication under dehydration condition, plants grown on FI_{SRS} (both soil column under well-irrigation) and DI (both soil column under deficit irrigation) were considered as representative controls for well-watered and drought conditions respectively while PRD (one wetted soil column, the drying remainder)-treated plants non-hydraulic signals are generated mainly from the drying roots and transported to shoot and water uptake is derived from wet roots to maintain plant water status and flux of sap.

2.3.1 Experimental conditions

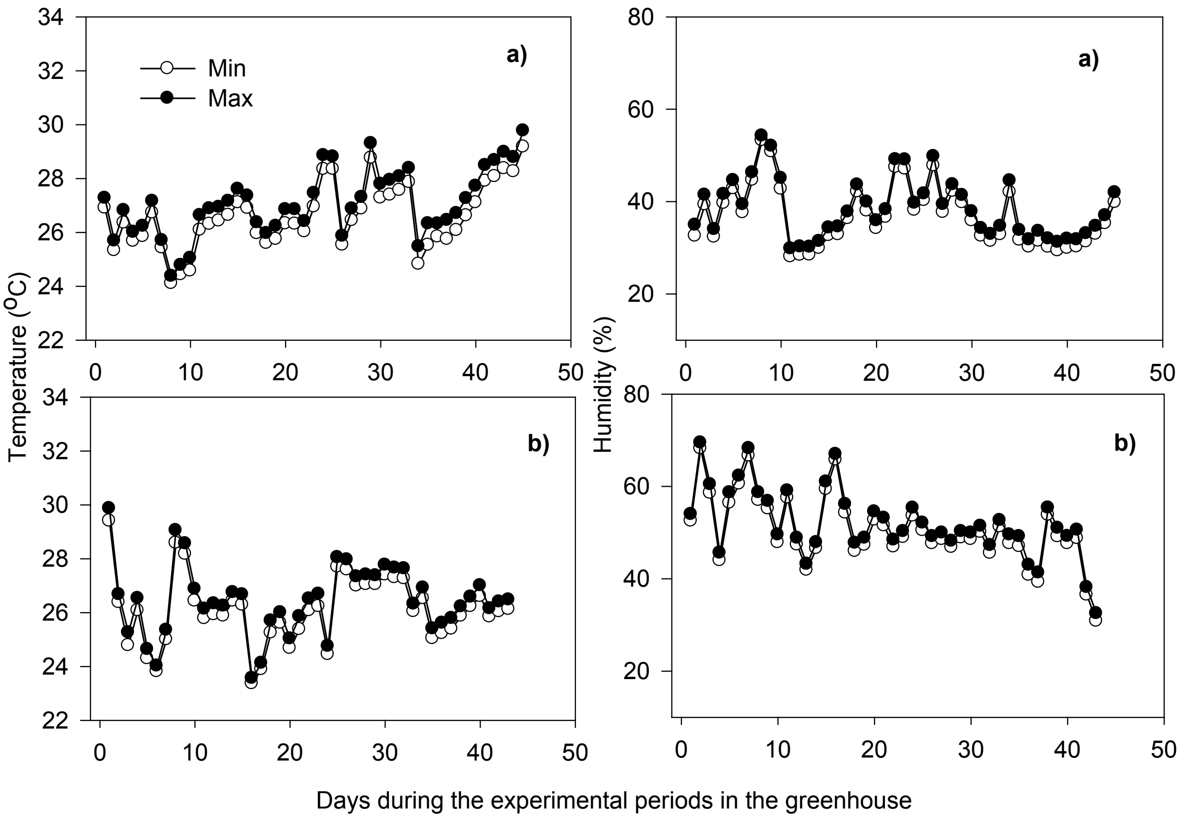


Fig. 2.5 The kinetics of temperature and humidity for the duration of the experiments

Note: split root system (SRS) experiment from 18.03-20.04 (a) and 17.09-17.10.2011(b).

Mikado grown on sandy soil, compost and Companero grown on compost were conducted from 18.03-20.04.1011 and Compost grown on sandy soil from 17.09-17.10.2011. From 18.03 to 20.04.1011, the mean minimum and maximum temperatures ranged from 24.11-24.37 and 29.17-29.77^{o}C, while minimum and maximum humilities were around 28% and 53% respectively (**Fig. 2.5 a**). The range of mean temperatures and humidity during the course of the last experiment, conducted from 17.09-17.10.1011, were about 23.37-29.87^{o}C and 30.24-69.43% (**Fig. 2.5 b**).

2.3.2 Experimental setup

***Cultivar:** Similar to drought spell experiment, two cultivars, Mikado and Companero, were used for four split root experiments on two cultivation media, the reason for selection was explained in detail in **section 2.2.2**.

* **Nutrient preparation:** to make nutrient solution for maize in SRS, 9 salt sorts used (**Table 2.4**). Each salt was diluted with 10 ml de-ionized water to produce ‘stock solutions’ and were stored in separate bottles. All stock solutions of salts were diluted with 40ml de-ionized water as well as 0.2% commercial nutrient liquid ‘Universal-Dünger’ before being applied; that contains 8% nitrogen, 8% P_2O_5, 6% K_2O, 0.01% B, 0.007% Cu, 0.013% Mn, 0.001% Mo, and 0.005% Zn. It is notable that difference in salt amount between control and Geohumus treatment explained in section 2.2.2 in detail.

Table 2.3 Nutrient supply for split root system experiments on sandy soil

Sort of salts	Amount of salt per plant (g)		stock solution (ml)	Periodic nutrient supply (week)			
				1-2	2-3	3-4	total used
	Control	Geohumus		Amount of stock solution (ml)			
NH_4NO_3	1.80	1.79	10	1	1.5	2	4.5
NaH_2PO_4*H_2O	4.96	4.96	10	1	1.5	2	4.5
K_2SO_4	2.63	2.61	10	1	1.5	2	4.5
$MgSO_4$*$7H_2O$	4.26	4.26	10	1	1.5	2	4.5
$C_{10}H_{12}Fe_2NaO_8$	0.16	0.16	10	1	1.5	2	4.5
$ZnSO_4$ $7H_2O$	0.02	0.02	10	1	1.5	2	4.5
$MnSO_4H_2O$	0.02	0.02	10	1	1.5	2	4.5
$CuSO_4XH_2O$	0.12	0.12	10	1	1.5	2	4.5
H_3BO_3	0.01	0.01	10	1	1.5	2	4.5

***Cultivation**

- **Pots and media preparation**: The SRS including two tubes with 3.5cm internal diameter, 20 cm height was supported by a PVC rectangle piece with 10 length and 6 cm width; all combined together by clue (**Fig. 2.6**). 30 SRS with plants and 12 without plants for both control and Geohumus treatment needed for one experiment; because xylem collection is very hard under dehydration, so unbalanced distribution in SRS number was designed e.g. 6, 10, and 14 SRS for FI, PRD, and DI respectively (**Table 2.4**). Two cultivation media including air-dried sandy soil (classified as ‘quaternary sand’ (Gerwin, Schillem et al. 2011) from Cottbus, Germany) and compost (produced at Hohenheim University) sieved (2mm) for four experiments with two cultivars (Mikado and Companero).

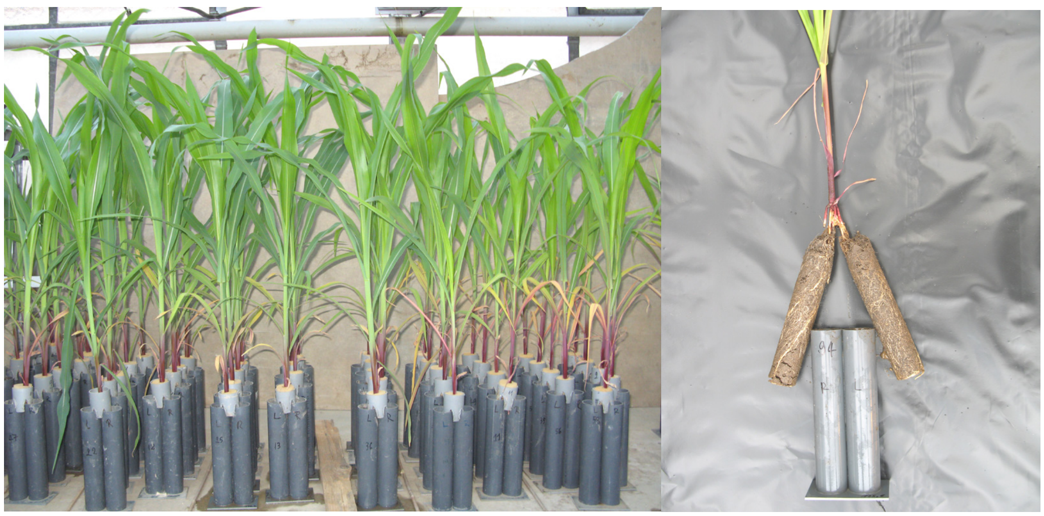


Fig. 2.6 Pots used for SRS experiment in the greenhouse

Table 2.4 Experimental designs in split root system

Cultivar	**Cultivation media**		**Pot number**		
	Type of media	**Amount (g)**	**FI_{SRS}**	**PRD**	**DI**
Mikado	Sandy soil	540	6	10	14
Companero	Compost	500			
Mikado	Compost	500			
Companero	Sandy soil	540			

Note: FI_{SRS}: full irrigation in split root sytem, PRD: partial root drying, DI: deficit irrigation

- **Seedlings**: Maize seeds were germinated and cultivated on trays filled with pure sandy with 10% stock solution of control (**Table 2.3**) under light high pressure sodium lamps (PL SON-K-400, DHlicht GmbH, Wülfrath, Germany) during 12 hours per day from 6 am to 18 pm under temperature 24-29^{o}C and humidity about 28-53%.

- **Transplantation:** a seedling having three leaves homogeneously selected was moved into SRS; roots were halved into two tubes of SRS with full water to make sure roots to be vertical with water volume. One day later, addition of cultivation medium was established after taking about 85% water in SRS out. Cultivation medium mixed with 10g Geohumus (Geohumus treatment) or without Geohumus (control) was also halved into SRS; amount of Cultivation medium depended on cultivation type, for instant, 540 g for sandy soil and 500g for compost (**Table 2.4**). Finally, water irrigation was carried again to ensure 100% field capacity by balance.

- **Nutrient supply**: to equalize nutrient for maize between control and Geohumus treatment, the SRS experiments, which utilized compost alone, were mixed some nutrients with control treatment because Geohumus contains nutrients e.g. 0.01g NH_4NO_3 and 0.02 g K_2SO_4 were added for per plant **(Table 2.3)**. The nutrients contained in compost were regarded as sufficient for the optimal development of maize. The treatments with a sandy soil/Geohumus mixture were supplied with nutrient stock solution with an interval of three days; the concentration of stock solution applied increased with the development of maize e.g. the concentration used for period of 1-2, 2-3, and 3-4 weeks were 1, 1.5 and 2 ml of stock solution respectively **(Table 2.3)**.

*** Watering**

Table 2.5 Soil moisture target (%) at onset for split root system experiments

Treatment	FI_{SRS}	PRD	DI
SS	14	10	7
SS + Geo	20	15	10
C	19	14	9
C + Geo	19	14	9

Note: FI_{SRS}: full irrigation, PRD: partial root drying, DI: deficit irrigation; SS: sandy soil, SS+Geo: sandy soil plus Geo, C: Compost, and C + Geo: compost plus Geohumus.

Water regimes for split root system experiments were based on an ‘exploratory experiment’ over 30 days; unique objective of this experiment is to observe change of soil moisture (SM) according to treatments. The result showed that SM in SS + Geo maintained over 30 days about 20% and was much higher than SS (14%) while C + Geo was stable 19% and equal C (without Geohumus) because Geohumus could hardly absorb water in compost medium; this was interpreted in section 4.1. So during from filling media into SRS to 28 days of age, SM on SRS experiments were maintained 100% field capacity (equal 14% for SM, 19% for compost) and replenished evapotranspiration daily by big balance (PCE-HPS60, capacity: 60kg ± 1g) for all split root systems (column FI_{SRS} of **Table 2.5**). At 28 days of age, soil moisture was treated according to water supply levels (**Table 2.5**) e.g. Three water supply levels (1) full water (FI), (2) Partial root drying (PRD): one side of SRS with FI, another side with 50% FI and (3) deficit irrigation (DI) with 50% FI for both sides

* **Sampling:** leaf samples and xylem sap (for ABA, water potential, osmotic potential, pH_{xylem}), and root water potential were carried out 40 hours after treating water. These progresses were described in section in detail 2.2.5, 2.2.6 and 2.2.7.

2.3.3 Plant analyses, plant water status, and gas exchange

Non-hydraulic signals, plant water status were determined as at section 2.2.5 and 2.2.6 respectively.

Regarding to gas exchange, we could not measure stomatal conductance (Gs) because of experimental condition but used root water potential (Ψ_{wroot}) to predict Gs based on equation generated from drought spell experiments. According to Palmer and O'Connell (2009), regression analysis could be determined relationship between single dependent (criterion) variable and on independent (predictor) variable to obtain a predicted value for the criterion resulting from a linear combination of the predictor. In our case, we also applied this tool to get Gs (predicted value) for slip root system experiments based on equation generated from the actual values of drought spell experiment. Although previous report showed that there was relationship leaf water potential (Ψ_{wleaf}) and Gs when a log-linear regression was applied to generate the curve and to get equation on *Eucalytus tetrodonta* (Prior, Eamus et al. 1997). However, we used Exponential Decay (Nonlinear Regression - Dynamic Fitting) with 200 of iteration from SigmaPlot software version 10.0 to generate the curves (best fit) and selected Ψ_{wroot} and Gs were considered as criterion and predictor respectively because the value of the coefficient of determination (R^2) between Ψ_{wroot} (predictor) and Gs (criterion) was much higher than Ψ_{wleaf} and Gs (Palmer and

O'Connell 2009) (**Table 7.15**). Further, mean of Ψ_{wroot} from between drought spell experiment and split root system was checked to make sure that no significant difference.

To avoid effect of treatment, Geohumus and control treatment on each cultivar (Mikado or Companero) were separately generated to get equation. We also assumed that relationship between Ψ_{wroot} and Gs of maize grown on sandy soil was consistent with grown on compost to obtain predicted values of Gs for experiments conducted on compost.

2.4 Statistical analyses

All experiments in this research were designed as completely randomized experiments. The influence of treatment factors were analyzed with the statistical software package SAS version 9.00 (SAS Institute Inc., Cary, NC, USA). There were three experimental groups in this research **(Table 2.6)**. Statistic analyses were explained in detail in the following:

* ANOVA of abiotic factors were performed with a one-factorial for sources of solutions (solution type), dose of nutrient solution (nutrient concentration) and two-factorial for temperature (temperature, nutrient solution), soaking time (time, nutrient solution), salt content (salt type, concentration), types of valance (valance type, concentration), incorporation depth (depth, nutrient) and used Geohumus from soil (Geohumus, soaking time), and Geohumus WHC after incubation in different salt solutions (salt sort, concentration). Another ANOVA of soil parameters was performed with two- factors for soil density (Geohumus, depth), soil suction (Geohumus, force).

* For drought spell experiments: soil moisture, growth, non-hydraulic signals, plant water status, and leaf gas exchange (Geohumus, water supply, harvesting point) ANOVA was performed with three-factors.

* For SRS experiments: we analyze data in the same way for these experiments. ANOVA of plant traits were carried out with three-factors for non-hydraulic signals, plant water status, leaf gas exchange (cultivation media, Geohumus and water supply).

Table 2.6 Summary of statistical analyses

Experiment	Factor No	Factors	Replica-tion
1. Impact of selected abiotic factors on Geohumus WHC and restorability			
Temperature	2	Temperature, nutrient solution	3
Soaking time	2	Time, nutrient solution	3
sources of solutions	1	Types of solution	3
Dose of nutrient solution	1	Nutrient concentration	3
- salt content	2	- Salt types, salt concentration	3
- types of valance	2	- Valance type, salt concentration	
incorporation depth	2	Depth, nutrient	9
Geohumus restorability			
- Used Geohumus from soil	2	- Geohumus source, soaking time	3
- Geohumus WHC after incubation in different salt solutions	2	- Salt sort, concentration	3
Soil density	2	- Geohumus, depth	18
Soil suction	2	- Geohumus, soil force	3
2. Drought spell (2 experiment)			
Soil and plant traits	3	- Geohumus, harvesting point,, harvesting points	3
***3.* Split root system experiment**	3	Cultivation media, water supply, Geohumus	5-11

Note: HP: 1-3, 4-5, and 1-5 indicate harvesting point from 1-3, 4-5, and 1-5 respectively.

Multiple comparisons of means were performed with the LSD test at alpha equal 5%. SigmaPlot 10.0 (Systat Software, Inc.) was used for the visual illustration of data.

3 RESULTS

3.1 Impact of selected abiotic factors on Geohumus WHC and restorability

3.1.1 Temperature

Water holding capacity (WHC) of Geohumus increased with increasing temperature for all tested solutions (**Fig. 3.1**). Distilled water absorption increased from 14 to about 20 ml g Geohumus^{-1} with gradually increased solution temperature from 10-50°C. For the tested temperature range and 10% nutrient solution Geohumus was only able to increase WHC about half compared to distilled water (from 10 to 13 ml g^{-1}) and only about 1 ml was additionally taken up in 100% nutrient solution (from 4 to 5 ml g^{-1}). With increasing concentration of nutrient solution WHC of Geohumus decreased strongly at all temperatures. At 20°C Geohumus in 100% nutrient solution absorbed only around 30% of the amount compared to distilled water.

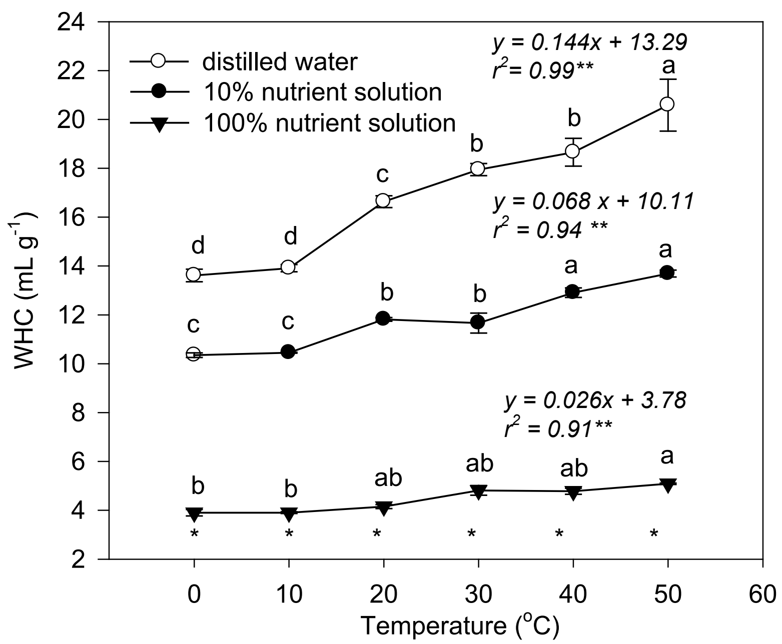


Fig. 3.1 Effect of temperature on Geohumus' water holding capacity in various media

Note: Different letter denotes significantly between temperature levels in the same treatment; star (*) marks for significant difference between media (distilled water, 10% and 100% nutrient solution) under the same temperature at alpha equal 5%; n=3.

3.1.2 Soaking time

WHC of Geohumus in de-ionized water continuously increased over a 60-hours incubation time from 15 ml g^{-1} to 30 ml g^{-1} **(Fig. 3.2)**. Geohumus WHC peaked in nutrient solution after 4 hours with 9 ml g^{-1}. WHC of Geohumus in nutrient solution was constant after 6 hours immersion time with 6 ml g^{-1}. The WHC of Geohumus in de-ionized water was between 2 (after 2 hours) and 5 times (after 60 hours) higher than in nutrient solution.

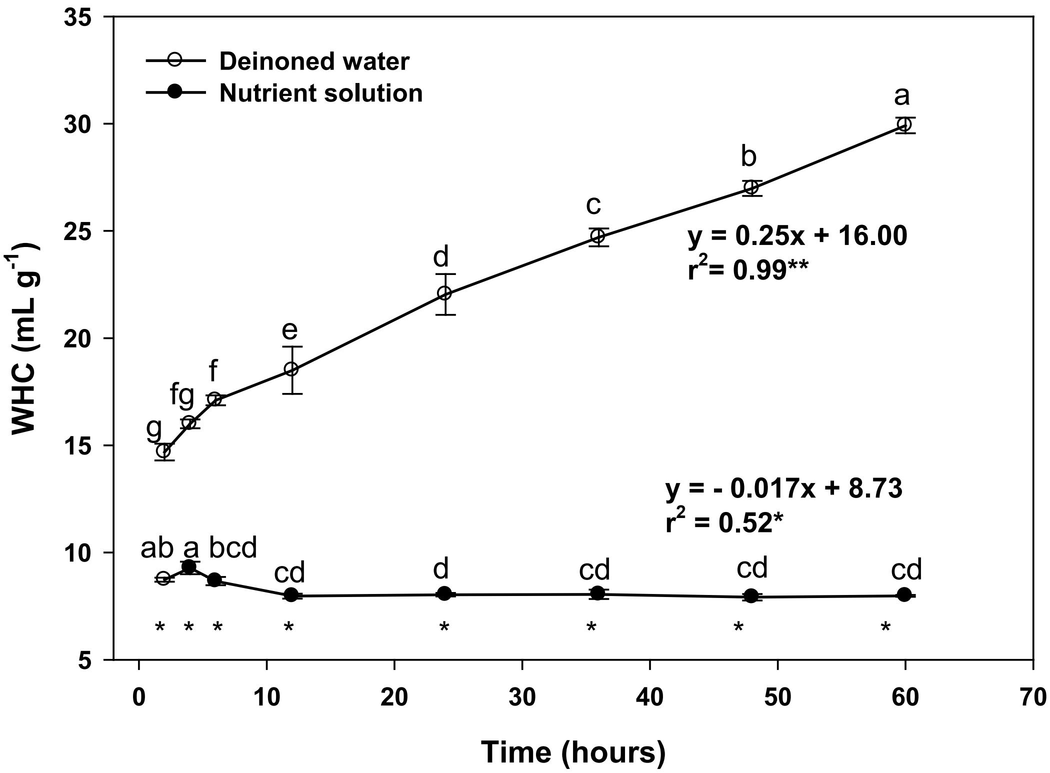


Fig. 3.2 Water holding capacity (WHC) of Geohumus immersed in deionized water and nutrient solution over a time period of 60 hours.

Note: Different letters and stars (*) indicate significant difference among time periods and media (nutrient solution and de-ionized water) respectively, at alpha equal 5%, n =3.

3.1.3 Various sources of solutions

The **Fig. 3.3** indicates the effect of individual soluble conditions on WHC of Geohumus. Geohumus immersed in distilled water had the highest WHC (13.53 ml g^{-1}), which differed considerably from the other solutions. WHC of Geohumus (10.86 ml g^{-1}) in tap water was also significantly higher than that in soil extract, compost extract, 100% nutrient solution and soil extract plus nutrient solution; no significant difference was observed between soil extract (8.16 ml g^{-1}), compost extract (7.87 ml g^{-1}), and 100% nutrient solution (7.73 ml g^{-1}), but all were considerably higher than soil extract mixed with stock solution (4.43 ml g^{-1}).

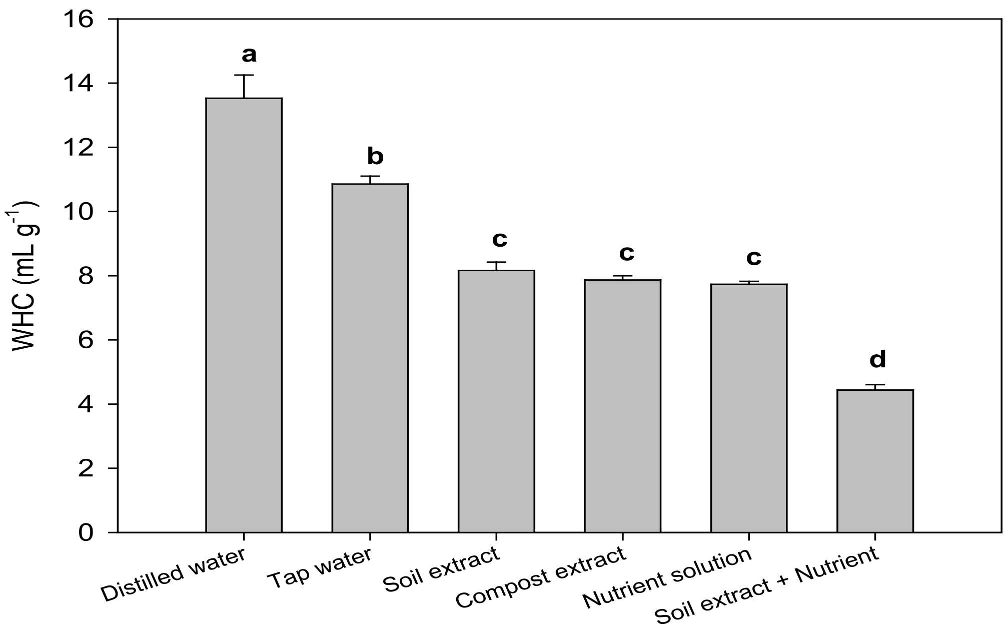


Fig. 3.3 Water holding capacity of Geohumus in variables of soluble media

Note: distilled water (1), tap water (2), soil extract (1:1) (3), compost extract (1:1) (4), nutrient solution (5) and soil extract (1:1) plus nutrient solution (6). Means with different letter denote significant difference at alpha 5%, n =3.

3.1.4 Concentration of nutrient solution

Fig. 3.4 indicates that when fertilizer is added to soil, it induces a negative effect on the WHC of Geohumus. The higher the nutrient concentration was, the lower the WHC of Geohumus. In fact, there were significant differences in WHC between the majority of doses of nutrient solution, except between 50 and 75% as well as 300% and 400%. However, WHC in 350% nutrient solution did not show a significantly distinct comparison to 400%.

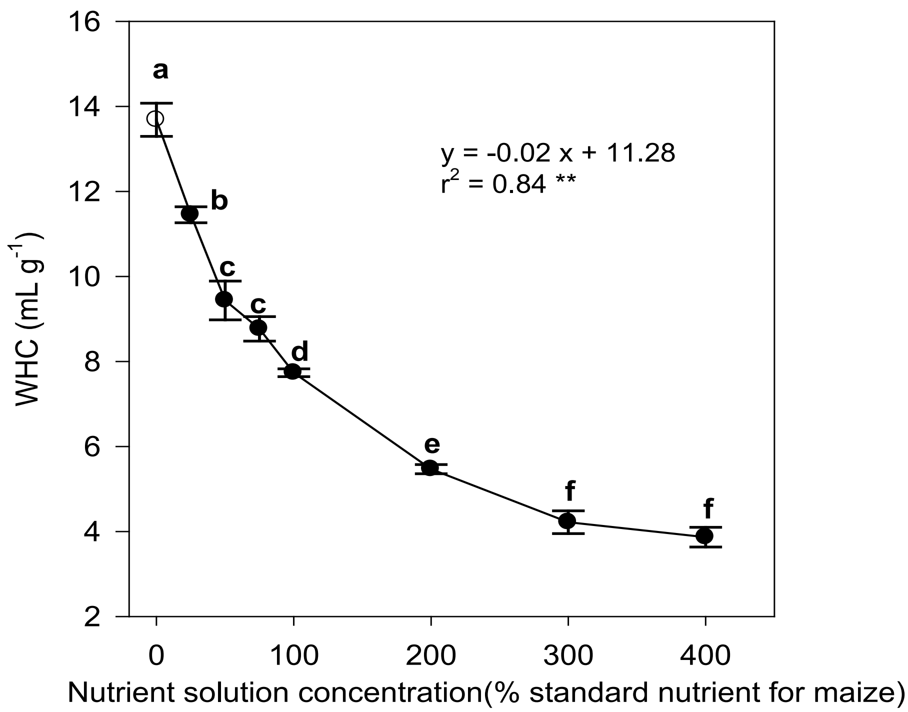


Fig. 3.4 Water holding capacity (WHC) of Geohumus in different nutrient solution concentrations

Note: Different letters indicate significant difference between doses at alpha equal 5%, n = 3.

3.1.5 Selected salts: salt content and types of valance

* Salt content

The figures shown in **Table 3.1** illustrate impact of salt types and salt concentrations on Geohumus WHC. Two specific salt solutions (KNO_3 and NH_4NO_3) were compared to estimate the effect of chemical elements on the WHC of Geohumus.

- Water absorption of Geohumus in KNO_3 and NH_4NO_3 solution was not significant different at any concentration, indicating that K^+ and NH_4^+ similarly affected the response of Geohumus.
- The comparison of NaCl and KCl, showed that K^+ ions impeded the absorption of water through Geohumus stronger than Na^+ ions. However, there was only a significant difference in water absorption under high concentrations (0.04-0.2M).

- Geohumus in KNO_3 solution absorbed more water than in KCl solution. This implies that the NO_3^- anion in solution impedes WHC of Geohumus less than the Cl^- anion.
- Surprisingly, at higher concentrations (0.05-0.2M), Geohumus showed higher water absorbing capacity at higher concentrations of K_2SO_4, compared to KCl; a behavior that is completely opposite when using higher concentrations (0.01-0.03M), with no significant difference at 0.04M.
- Mg^{2+} from $MgSO_4$ solution was comparable to K^+ from K_2SO_4 solution, regarding the reduced expansion of Geohumus. This difference may be explained through the impact of cation-valence.
- There were exceeded gap in WHC when Geohumus was immersed into $MgSO_4$ and $FeSO_4$ solution. Considering about chemical property, Fe has acidity while Mg is alkaline. This distinction depends on the chemical properties rather than valence, as Fe and Mg are both bivalent.
- $Al_2O_{12}S_3$ is one of the compounds which can impede WHC of Geohumus the most. In this trial, it is just weaker than the compound $FeSO_4$, this difference was seen in a dose-range of 0.1-0.2 M.

WHC of Geohumus correlated negatively with increasing doses of mixed salts (**Table 3.2**). The level of relationship is shown in **Table 3.1** where most salts illustrated negative correlation, except $FeSO_4$. All concentrations of mixed salt were close to $FeSO_4$ or $MgSO_4$ solutions; this indicates that, in comparison to other salts, iron and magnesium salts reduce water absorption of Geohumus. In other words, the presence of these ions strongly obstructs the expansion of Geohumus water absorption in soluble media.

Geohumus in de-ionized water absorbed more than 13 ml g^{-1}. When the salt concentration was increased to about 3g L^{-1}, the WHC for immersed Geohumus decreased to around 8 ml g^{-1} and less 6 ml g^{-1} for the group of compounds with valence I (K_2SO_4, KCl, and NaCl) and valence II ($MgSO_4$ and $FeSO_4$) respectively (**Table 7.1**). Even within the same group, there was a significant difference in water absorption between compounds with the elements, Iron (Fe) and Magnesium (Mg). Salt concentration about 6 g L^{-1}, there was not significant difference between K_2SO_4, NaCl and KCl while a considerable difference between Fe and Mg was maintained. There was no remarkable change in WHC within the same group at salt concentrations higher than 7g L^{-1}. In valence II group, WHC was fairly constant, although salt concentrations reached approximately 15 g L^{-1} while WHC in valence I group decreased continuously.

Table 3.1 Regression between concentrations of compound and water absorption of Geohumus

Compounds	**R^2**
KNO_3	0.82*
NH_4NO_3	0.88*
NaCl	0.93**
KCl	0.95**
K_2SO_4	0.91**
Mixed	0.95**
$MgSO_4$	0.74*
$Al_2O_{12}S_3$	0.78*
$FeSO_4$	0.59ns

*Note: *, ** denote 5%, 1%, ns = non- significance*

*** Types of valence**

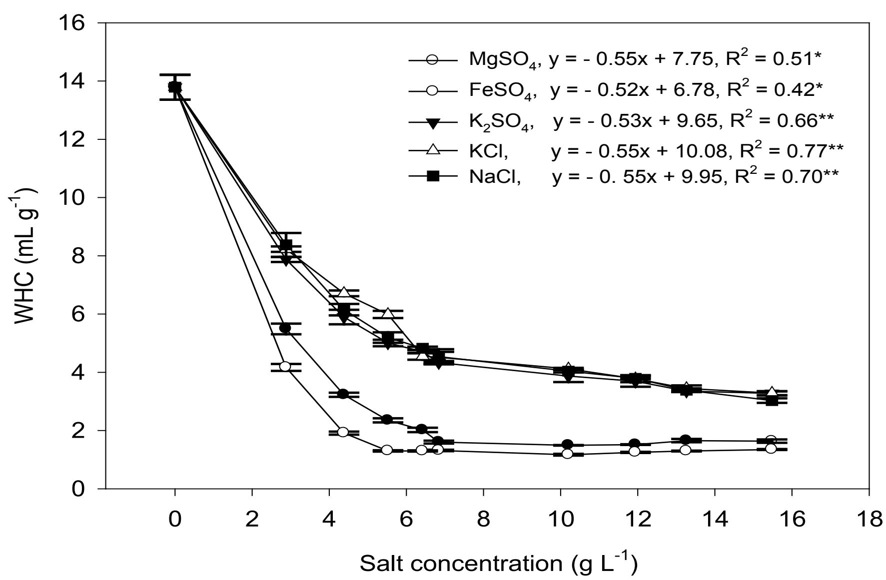


Fig. 3.5 Relationships between salt concentration of different compounds and water holding capacity (WHC) of Geohumus

Note: Different letters denote significantly between doses at alpha equal 5%, n = 3.

Table 3.2 Impact of types and concentration of chemical compounds on water holding capacity of Geohumus (ml g^{-1})

Con. (M)	KNO_3 (1)	NH_4NO_3 (2)	NaCl (3)	KCl (4)	K_2SO_4 (5)	$MgSO_4$ (6)	$FeSO_4$ (7)	Mixed (8)	$Al_2O1_2S_3$ (9)
0.20	1.92 a F	1.98 a G	1.76 a F	1.35 b F	1.85 a F	0.62 c D	0.13 d C	0.52 c F	0.14 d E
0.15	2.18 ab F	2.12 ab G	2.26 a F	1.37 c F	1.88 b F	0.55 d D	0.13 e C	0.54 d F	0.14 e ED
0.10	3.36 b F	2.89 a F	3.15 a E	1.77 c F	2.04 bc F	0.49 ed D	0.13 e C	0.75 d F	0.24 e ED
0.05	4.04 a E	3.80 a E	3.20 b E	2.45 c F	2.62 c E	0.73 e D	0.13 f C	1.32 d E	0.27 f ED
0.04	4.06 a E	4.08 a E	3.98 a D	3.43 b E	3.48 b D	1.01 d CD	0.27 e C	2.09 c D	0.44 e ED
0.03	4.74 a D	4.77 a D	4.38 ab D	4.25 b D	3.75 c CD	1.41e C	0.28 f C	2.85 d C	0.62 f CD
0.02	5.89 a C	5.88 a C	5.21 b C	4.91 b C	4.16 c C	1.61e C	0.36 g C	3.34 d C	0.96 f C
0.01	7.91 a B	7.73 a B	6.70 b B	6.63 b B	5.42 c B	3.42d B	1.11 f B	4.88 c B	1.98 e B
0.00	13.5 A	13.5 A	13.5 A	13.5 A	13.5 A	13.5 A	13.5 A	13.5 A	13.5 A

Note: Con.: concentration; Mixed: mixing solutions 3, 4, 5, 6 and 7 together with respective concentration. Normal letter and capitalized letter compared in row and column respectively. Means with the same letter are not significantly different at Alpha equal 5%, n = 3.

3.1.6 Incorporation depth

Table 3.3 gives an indication of the influence of soil pH and EC in different soil layers on the water absorption capacity of Geohumus. Values of EC in control as well as treatment at saturation-point show a decreasing trend from top to bottom. The application of nutrients causes the EC of the control-treatment to be lower than that of treatments throughout the four layers. There were significant differences in the top (0-7.5cm) and fourth (32.5-45cm) layers.

Table 3.3 Impact of EC and pH in different soil layers of pot on WHC of Geohumus

Layers (cm)	Soil EC (µmhos cm^{-1})		Soil pH		WHC (ml g^{-1})	
	Control	**Treatment**	**Control**	**Treatment**	**Control**	**Treatment**
0-7.5	73.7 b C	93.3a B	7.12 b A	7.58 a A	8.00 a A	8.44 a A
7.5-20	82.6 a B	89.2a B	7.19 b A	7.54 a A	7.00 a B	6.44 a B
20-32.5	8.73 a B	89.4 a B	7.23 b A	7.51 a A	5.89 a C	5.00 b C
32.5-45	106.4 b A	132.8a A	7.16 a A	7.16 a B	5.56 a D	3.33 b D

Note: Normal letter and capitalized letter compared in row and column respectively. Means with the same letter are not significantly different at Alpha equal 5%, n = 9. Control: not mixing with nutrient solution and treatment mixing with solution.

3.1.7 Geohumus WHC restorability

*** used Geohumus (from cultivation media)**

Fig. 3.6 shows that the restorability of used Geohumus had no difference between 4 soil layers as well as between two soaking time levels (2 and 24 hours). Geohumus mixed with compost led its restorability reduced significantly compared to that mixed with sandy soil but no significant difference compared to Geohumus from 4 layers immersed 2 and 24 hours as well,

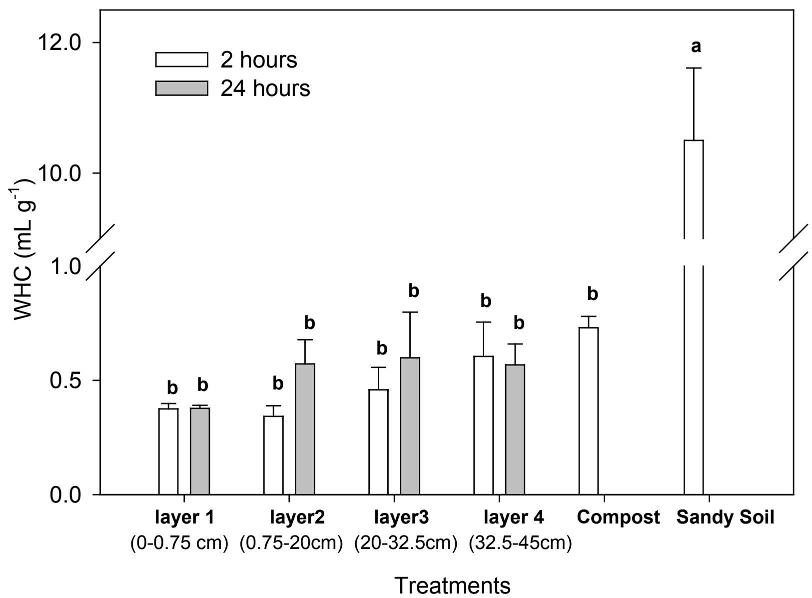


Fig. 3.6 Water holding capacity (WHC) of used Geohumus after one crop of maize at various sandy soil layers of pots

Note: sandy soil layer 1-4 applied 100% foundation fertilizer; on sandy soil of split root system (nutrient solution regularly applied); and compost (no nutrient was applied) with difference length of imbedding time in solution (2 and 24 hours). The same letters are not significant difference in WHC between two hours and 24 hours at alpha equal 5%.

*** Used Geohumus (from soluble media)**

Table 3.4 Water capacity of Geohumus (imbedded in chemicals) after washing and imbedding in distilled water for 6 hours

Salt concentration (M)	**$FeSO_4$ (1)**	**$Al_2O_{12}S_3$ (2)**	**KNO_3 (3)**	**NH_4NO_3 (4)**
0.2	0.05b B	0.03b D	11.35a D	11.50a E
0.15	0.11b B	0.12b CD	12.32a CD	12.33a E
0.10	0.15b B	0.35b CD	13.26a C	14.58a CD
0.05	0.15c B	0.63c CD	13.29b C	14.95a CD
0.04	0.21d B	1.05c C	16.28a B	15.47b BC
0.03	0.24b B	1.10b C	16.41a AB	15.70a BC
0.02	0.52b B	2.20b B	17.20a AB	16.28a AB
0.01	3.59c A	7.70b A	18.06a A	17.67a A

Note: *normal letter and capitalized letter compared in row and column respectively. Means with the same letter are not significantly different at Alpha = 5%. n = 3.*

Table 3.4 shows the results of Geohumus restorability for different salt solutions. In higher concentrations of salt, the ability to restore it was lower. The negative effects of salt concentration still remained even though Geohumus was washed with distilled water to mitigate the absorption of salts or ions. Additionally, there were differences in restorability of Geohumus between valance group I and II, with group I having a higher restorability than group II. The restorability of Geohumus depended on the properties and concentrations of specific salt solutions in which Geohumus was immersed. Salt solutions at higher and stronger concentrations resulting in lower Geohumus restorability.

3.1.8 Soil bulk density, suction and moisture as influenced by treatments

a) Soil bulk density

Table 3.5 Soil bulk density (g cm^{-3}) under drought spell experiment

Layers (cm)	**Mikado**		**Companero**	
	Sandy soil	**Geohumus**	**Sandy soil**	**Geohumus**
0.0-0.75	1.58a B	1.62a B	1.53a B	1.61aB
0.75-20	1.93a A	1.87a A	1.78a A	1.76a AB
20-32.5	1.92a A	1.87a A	1.85a A	1.85a A
32.5-45	1.97a A	1.95a A	1.83a A	1.90a A

Capitalized and normal letters indicate significant differences at alpha = 5%, (n= 18) within one column and between sandy soil and Geohumus treatment in the same cultivars.

BD mean measured at all plant harvest times ranged from 1.58 -1.97g cm^{-3} (sandy soil) and 1.62-1.95 g cm^{-3} (Geohumus treatment) for Mikado, and about 1.53-1.85 g cm^{-3} (soil) and 1.61-1.90 g cm^{-3} (Geohumus) for Companero (**Table 3.5)**. There was no difference in soil bulk density for both Mikado and Companero, with or without Geohumus in soil depths below 0.75 cm. There were significant differences in soil bulk density between the top layers and the lower layers. Secondly, there was no significant difference in soil bulk density between control (soil) and soil + Geohumus at any layer. The used cultivars Mikado and Companero had no influence on soil bulk density.

b) Soil matrix potential

Table 3.6 Impact of Geohumus on relationship between soil matrix potential and water content (g water g^{-1} soil).

pF	MPa	Water content	
		Sandy Soil	SS + Geohumus
1	-0.001	0.52a A	0.52a A
1.8	-0.009	0.44a AB	0.47a AB
2.5	-0.083	0.38a B	0.41a B
4.2	-1.60	0.02a C	0.03a C

Note: *normal letter and capitalized letter compared in row and column respectively. Means with the same letter are not significantly different at Alpha = 5%. n = 3.*

Table 3.6 shows soil water content with change of pF value or applied pressure. Both for sandy soil (SS) and sandy soil plus Geohumus (SS + Geo), the water content regularly decreased with increasing pF value. At wilting point (pF = 4.2), the water content of SS and Geo + SS were 0.02 and 0.03 g water $soil^{-1}$ respectively; there was no significant difference in water content between SS and SS + Geo.

In short, the impact of selected abiotic factors on Geohumus WHC and restorability were summarized following:

- There was positive relationship between WHC of Geohumus and temperature. However, WHC showed a dramatic increase when Geohumus was soaked in low nutrient solution only.
- Geohumus WHC in deionized water dramatically increased during 60 hours while in nutrient solution increased within 4 hours. There was negative correlation between nutrient concentration and Geohumus WHC.
- Different solution sources led to difference in WHC of Geohumus
- Within range of 0-0.2 M, Geohumus WHC showed closely negative correlation with concentration with majority of selected salts (KNO_3, NH_4NO_3, NaCl, KCl, K_2SO_4, $MgSO_4$, $AlO_{12}S_3$) but not $FeSO_4$. The group of salts with a valance II reduced WHC of Geohumus stronger than valance I. Different ionic sources had

different hindrance in Geohumus WHC in the decreasing order: Fe^{2+}>Al^{3+}>Mg^{2+}>Na^{+}>NH_4^{+} ≈K^{+}.

- Similar to soluble media, WHC of Geohumus in soil medium was also affected by salt concentration
- Geohumus could almost not absorb water when it was mixed in soil with base nutrient or in Compost but the divided application of fertilizer led to remarkably improve its WHC.
- The restorability of WHC of Geohumus in soluble media is significantly reduced in higher salt concentrations or stronger ions.
- Geohumus had no significant effect on soil bulk density and soil suction as well.
- Geohumus application to sandy soil did not improve mean soil moisture and soil moisture in separate layers under drought spell and full irrigation as well; even in Mikado the soil moisture in the first two layers with Geohumus application under full irrigation was significantly lower than in control pots.

3.2 Influence of Geohumus on morphological and physiological responses of two maize cultivars under prolonged water deficit

3.2.1 Soil moisture

Under constant full irrigation Companero showed no difference in mean soil moisture of four separated layers, irrespective of Geohumus was mixed into the sandy soil or not (**Fig. 3.7**). However, at day 8 mean soil moisture of Mikado with Geohumus (9.8 % g g^{-1}) was even significant lower compared to the control (10.5% g g^{-1}). In the drought spell treatment, mean soil moisture of both cultivars decreased within eight (Mikado) and nine (Companero) days after irrigating was decreased to 2% g g^{-1}. Geohumus had no effect on the soil dry-out dynamics and also no difference on soil moisture after rewetting which was measured on day 15.

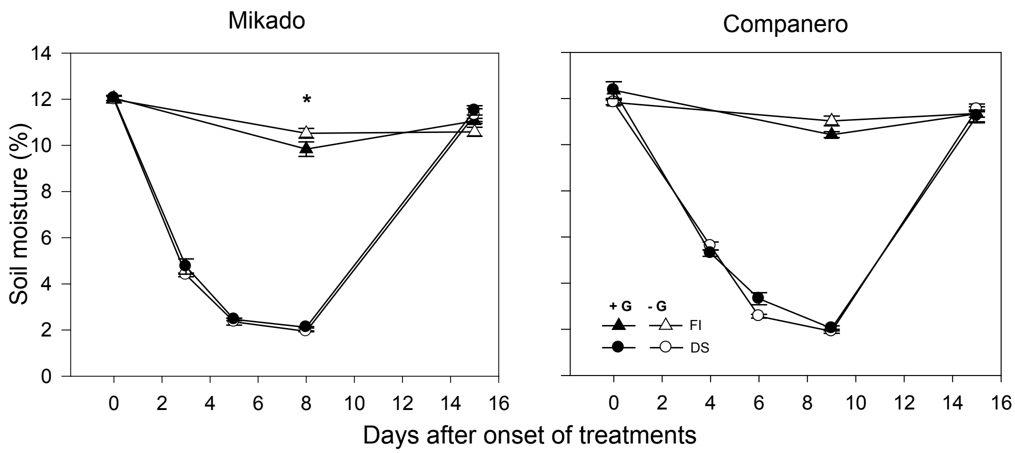


Fig. 3.7 Mean soil moisture of four soil layers in pots of the two maize cultivars Mikado and Companero as influenced by Geohumus application under full irrigation and progressive drought

Note: FI: full water; DS: drought spell; -G: sandy soil; and +G: sandy soil plus Geohumus. Significant difference between control and Geohumus under FI (*) or DS (+) condition at alpha = 5%, n = 3. Error bars = standard deviation.

3.2.2 Growth

a) Green leaf area

Under full irrigation, green leaf area of both cultivars was more than doubled during 15 days of the experiment and Geohumus significantly increased the leaf area at day 8 compared to the control treatment, however, at day 15 the effect of Geohumus was not significant anymore (**Fig. 3.8 a**). Under drought spell, leaf area decreased a few days after irrigation was withheld but strongly increased after rewetting with almost the same level as full irrigation treatments at day 15. Geohumus application resulted in leaf growth of Mikado for two more days compared to control resulting in a significant higher leaf area at day 5. However, on day 8 and after rewetting Geohumus could not increase leaf area compared to control. No Geohumus effects under drought were measured for the Companero cultivar, but after rewetting, leaf area of the Geohumus treatment was significantly higher.

b) Leaf dry weight (LW)

Leaf dry weight under full irrigation of both cultivars on control and Geohumus treatment showed an increase under full irrigation throughout the period of observation but that under drought spell were little fluctuated at day 8 (Mikado) and day 9 (Companero) (**Fig. 3.8 b**). Leaf dry weight of Mikado on Geohumus (13.7 g) under full irrigation on day 8 was superior to control (9.5 g) while Companero on Geohumus seemed slightly higher compared to control after onset. Turning to drought spell, Mikado leaf dry weight on Geohumus (9.9 g) showed significantly higher than control (8.2 g) at day 5.

c) Shoot dry matter

Fig. 3.8 c illustrates the increasing of shoot dry matter weight over the experimental period of 15 days. Drought decreased shoot dry matter for the Mikado cultivars after 15 days, but not for the Companero compared to the control. While the drought treatments Geohumus had no effect on shoot dry matter, the application of Geohumus under full irrigation increased shoot dry matter at day 8 and 15 compared to control.

d) Total root weight

Root weight under full irrigation of both Mikado and Companero was higher with Geohumus compared to control. However, the drought treatment showed the opposite result (**Fig. 3.8 d**). Mikado and Companero total root weight of all treatments increased during period of observation, except that Companero under drought spell, which showed a decrease on day 9. Companero total root weight on control at onset and on day 6 under drought spell was considerably higher with Geohumus at the corresponding sampling days. However, significant difference between control (4.3 g) and Geohumus (5.5 g) under constant full irrigation was only found at day 9. Similarly, Mikado total root weight with Geohumus (4.9 g) at day 8 under full irrigation was also higher compared to the control (3.4 g).

e) Root weight density

Root weight density of both cultivars at onset on control under full irrigation was higher than Geohumus treatment, showing significant differences in that of Companero in layer 1, 2. However, root weight density of these cultivars with Geohumus tended higher than the control in following days; considerable difference in Companero root weight density in layer 3 at day 9 was seen (**Fig. 3.9**). Turning to drought spell condition, most cases of root weight density of Mikado and Companero in majority of cases in four layers on control seemed higher than Geohumus treatment. However, significant distinctions in root weight density of Mikado on day 5 in layer 1, day 15 in layer 2 and of Companero at onset in layer 1, day 6 and 15 in layer 2, and day 6 in layer 4 were observed (**Fig. 3.10**).

f) Root length density

At onset, root length density of both cultivars on control seemed higher Geohumus at all layers; significant difference in that of Companero in layer 3-4 was found (**Fig. 3.11**). However, Mikado (day 8) and Companero (day 9) root length density on Geohumus in the last two layers under full irrigation was likely higher than control, showing significant difference on Mikado in layer 4 and Companero in layer 3 on day 9 and 15; contrary, it showed the opposite result in the first two layers. During the observation period, Mikado and Companero root length density on control under drought treatment tended higher than Geohumus treatment. However, significant difference in Companero root length density in layer 1 at day 9 and layer 4 at day 15 were noted.

g) Vertical root distribution

Geohumus application mediated early Mikado root penetration of the lowest layers under both full irrigation and drought compared to the control (**Table 3.7**). However, Companero was not changed as influenced by Geohumus application and water regimes as well. The roots of Companero tended to develop into the first two layers, while the roots of Mikado distributed to the lowest layer as the experiment continued.

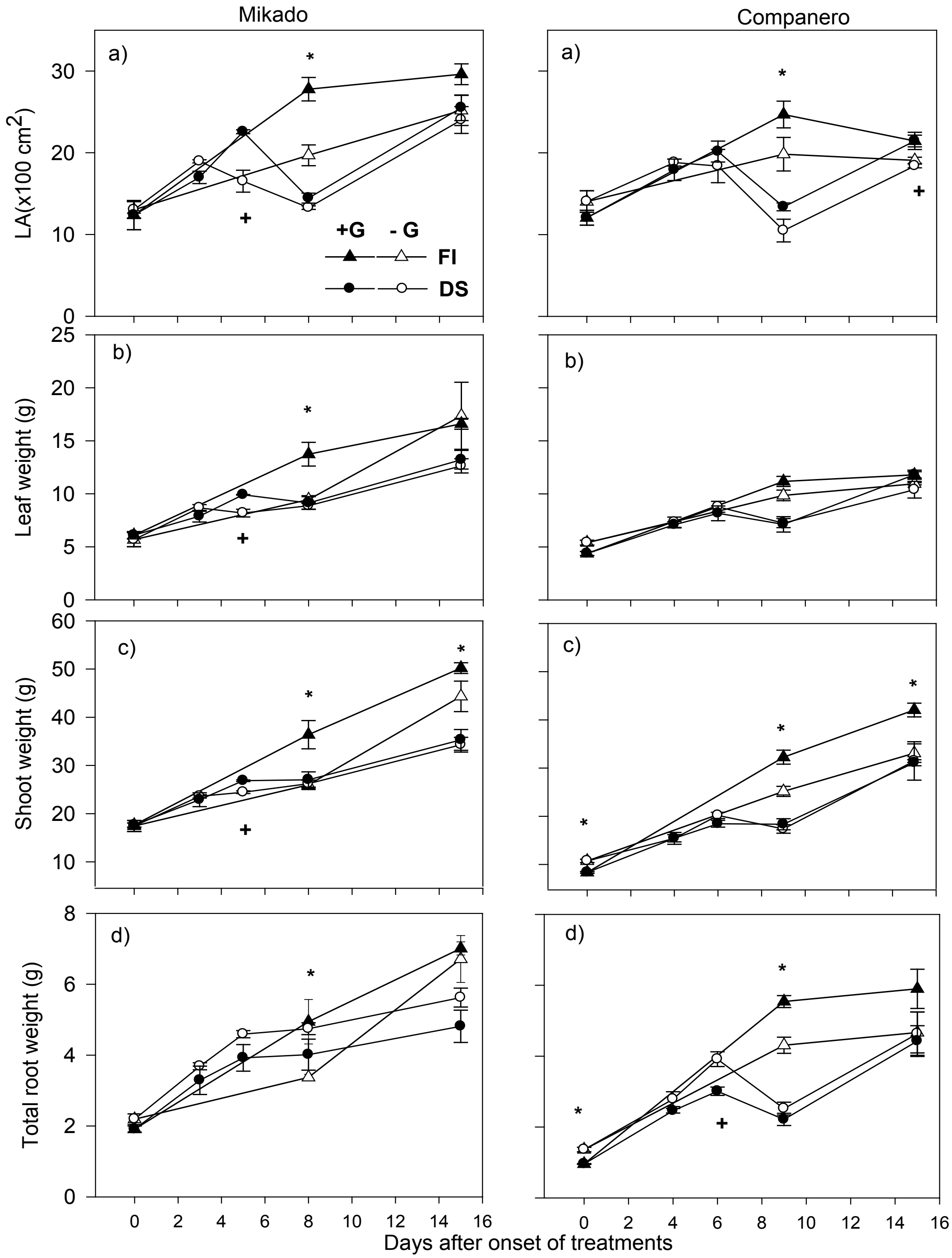


Fig. 3.8 Leaf area (a), leaf weight (b), shoot weight (c), and total root weight (d) of the two maize cultivars Mikado and Companero as influenced by Geohumus application under full irrigation and progressive drought.

Note: FI: full irrigation; DS: drought spell; -G: sandy soil; and +G: sandy soil plus Geohumus. Significant difference between control and Geohumus under FI (*) or DS (+) condition at alpha = 5%, n = 3. Error bars = standard deviation.

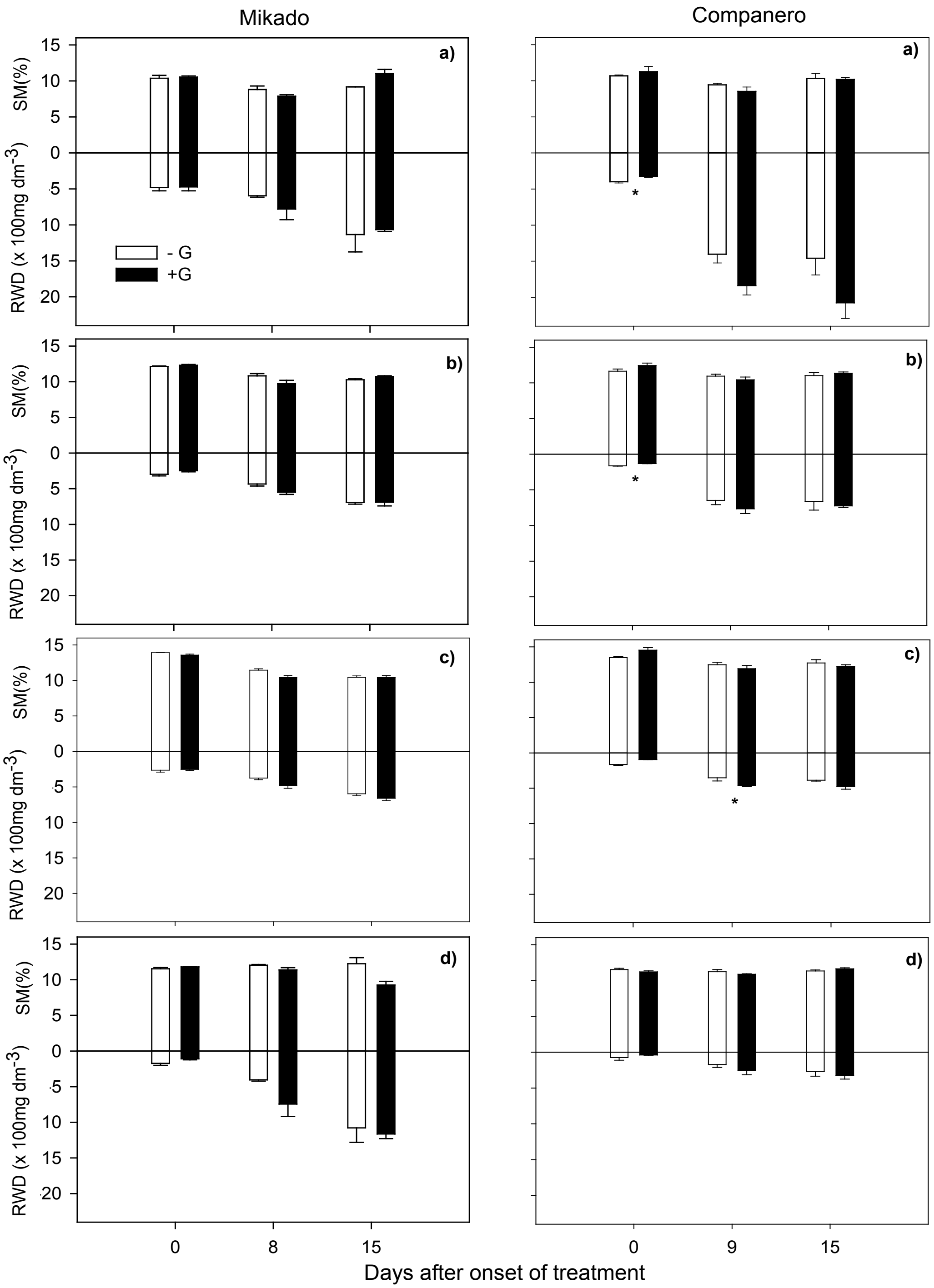


Fig. 3.9 Soil moisture (SM) and root weight density (RWD) distribution of two maize cultivars Mikado and Companero as influenced by Geohumus application under full irrigation

Note: Control (-G) and Geohumus treatment (+G); four layers of potted soil profile (0-7.5 (a); 7.5-20 (b); 20-32.5 (c) and 32.5-45 cm (d).). Significant difference between control and Geohumus under FI (*) condition at alpha = 5%, n = 3. Error bars = standard deviation.

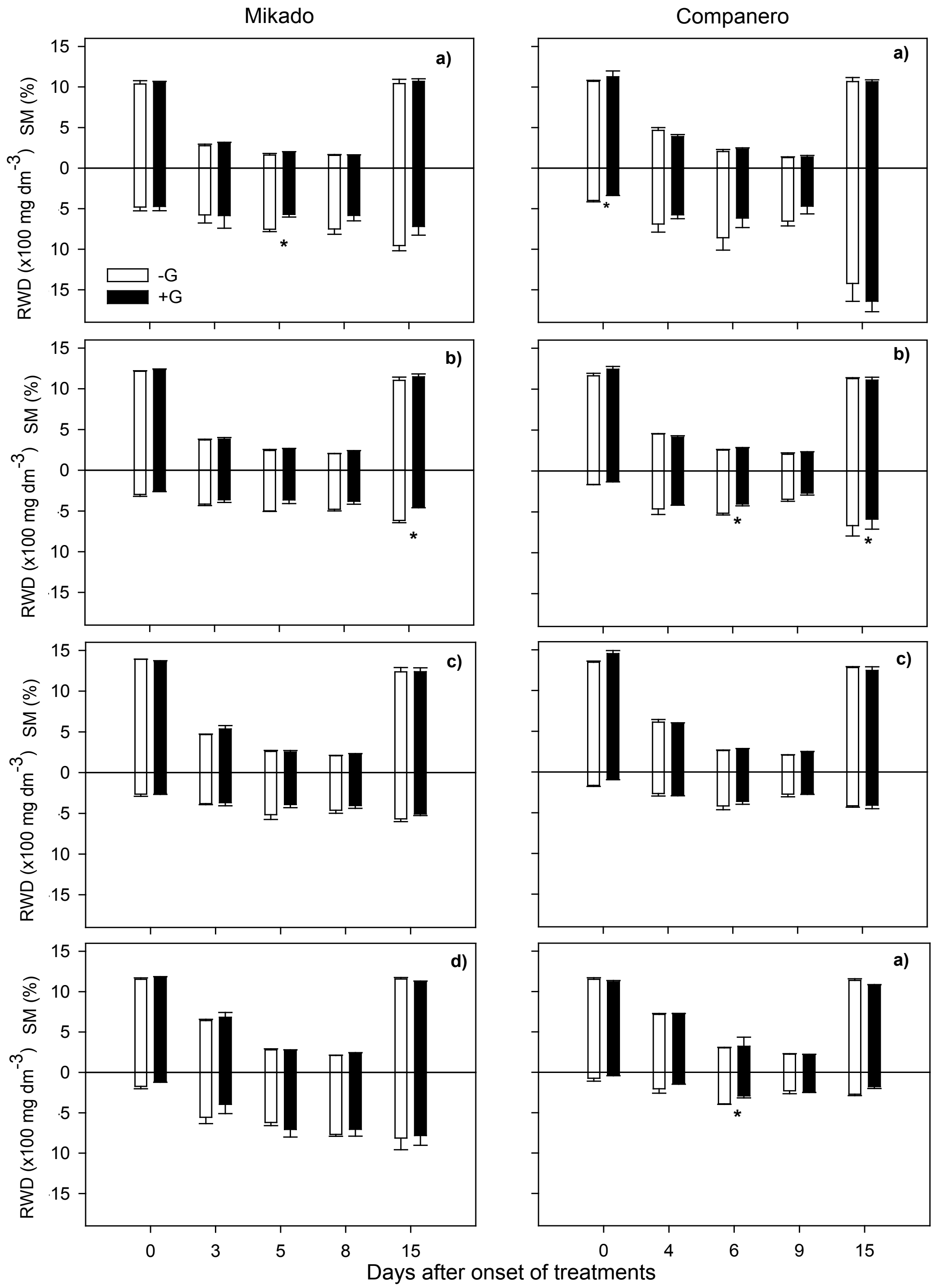


Fig. 3.10 Soil moisture (SM) and root weight density (RWD) distribution of two maize cultivars Mikado and Companero as influenced by Geohumus application under progressive drought

Note: Control (-G) and Geohumus treatment (+G); four layers of potted soil profile (0-7.5 (a); 7.5-20 (b); 20-32.5 (c) and 32.5-45 cm (d). Significant difference between control and Geohumus under DS (+) condition at alpha = 5%, n = 3. Error bars = standard deviation.

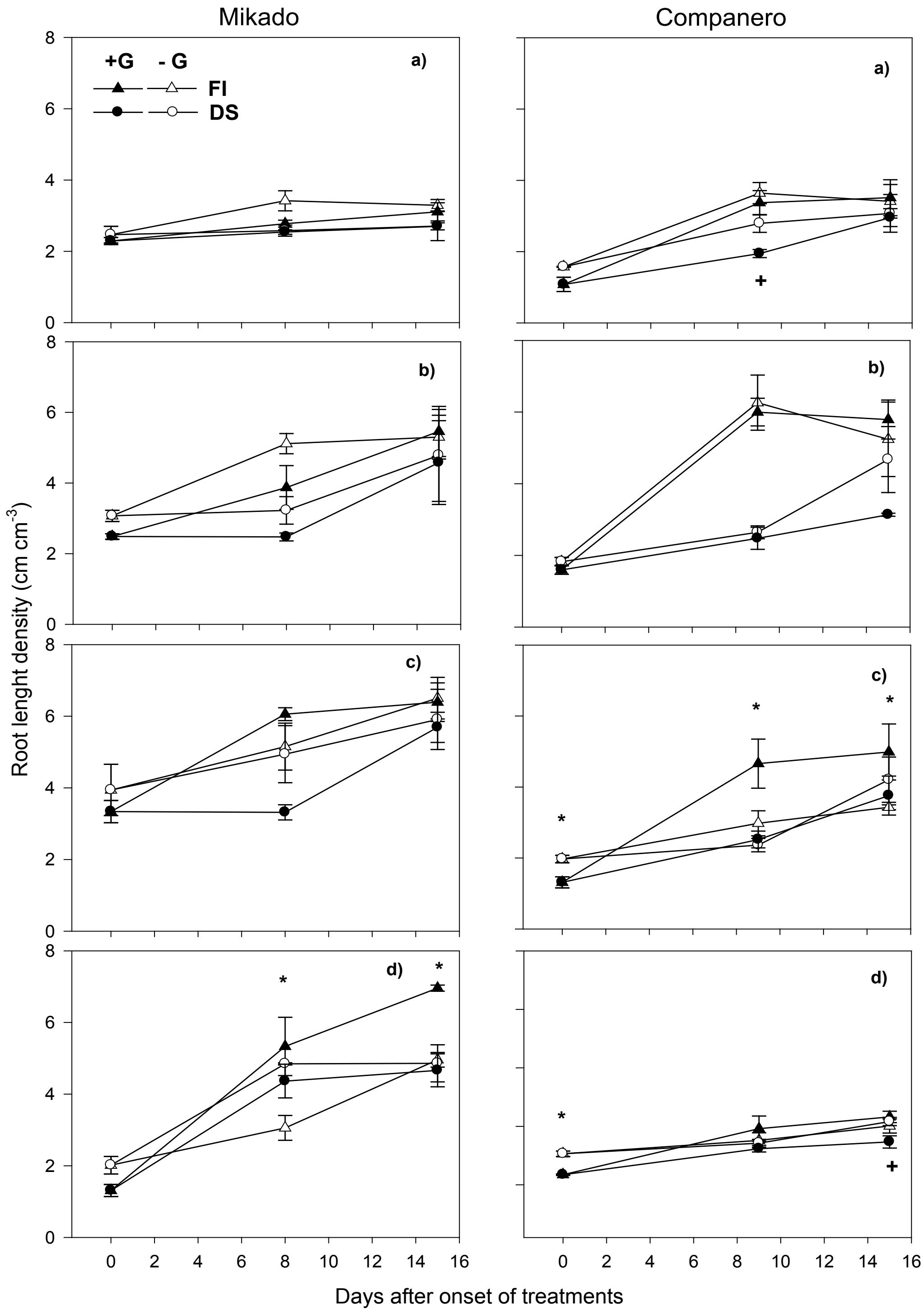


Fig. 3.11 Root length density (RLD) distribution of two maize cultivars Mikado and Companero as influenced by Geohumus application under full irrigation and progressive drought

Note: Control (-G) and Geohumus treatment (+G); four layers of potted soil profile (0-7.5 (a); 7.5-20 (b); 20-32.5 (c) and 32.5-45 cm (d). Significant difference between control and Geohumus under FI (*) or DS (+) condition at alpha = 5%, n = 3. Error bars = standard deviation.

Table 3.7 Vertical root distribution (%) of potted soil profile of two maize cultivars Mikado and Companero during observations

Cultivars	Water regimes	Soil layer depth (cm)	Days after onset of treatments									
			Control					Geohumus treatment				
			0	3	5	8	15	0	3	5	8	15
Mikado	Full Irrigation (FI)	0-7.5	40a			33a	32a	44a			31a	30a
		7.5-20	24b			24b	20b	23b			22ab	19b
		20-32.5	22b			21b	17b	23b			19b	18b
		32.5-45	14c			22b	31a	10c			29ab	32a
	Drought Spell (DS)	0-7.5	40a	30a	32a	31a	32a	44a	34a	28a	29a	29a
		7.5-20	24b	21a	21c	19b	21bc	23b	21a	18b	18b	18b
		20-32.5	22b	20a	22bc	19b	19c	23b	22a	19b	19b	21b
		32.5-45	14c	29a	26b	31a	28ab	10c	23a	35a	34a	32a
			0	4	6	9	15	0	4	6	9	15
Compan-ero	Full Irrigation (FI)	0-7.5	50a			55a	53a	55a			55a	58a
		7.5-20	21b			25b	24b	22b			23b	20b
		20-32.5	21b			14c	14c	16c			14c	13c
		32.5-45	9c			7d	10c	7d			8c	9d
	Drought Spell (DS)	0-7.5	50a	42a	39a	44a	51a	55a	41a	37a	38a	58a
		7.5-20	21b	29b	24b	23b	24b	22b	29b	24b	22b	21b
		20-32.5	21b	16c	19b	18b	15c	16c	20c	22b	21b	15c
		32.5-45	9c	13c	18b	15b	10c	7d	10d	17b	20b	6d

Note: different letters denote significant difference in percentage of root distribution among four layers of potted soil profile under the same water regimes and sampling days at alpha = 5%, n = 3.

3.2.3 Hydrophilic polymer effects on plant root-shoot partitioning

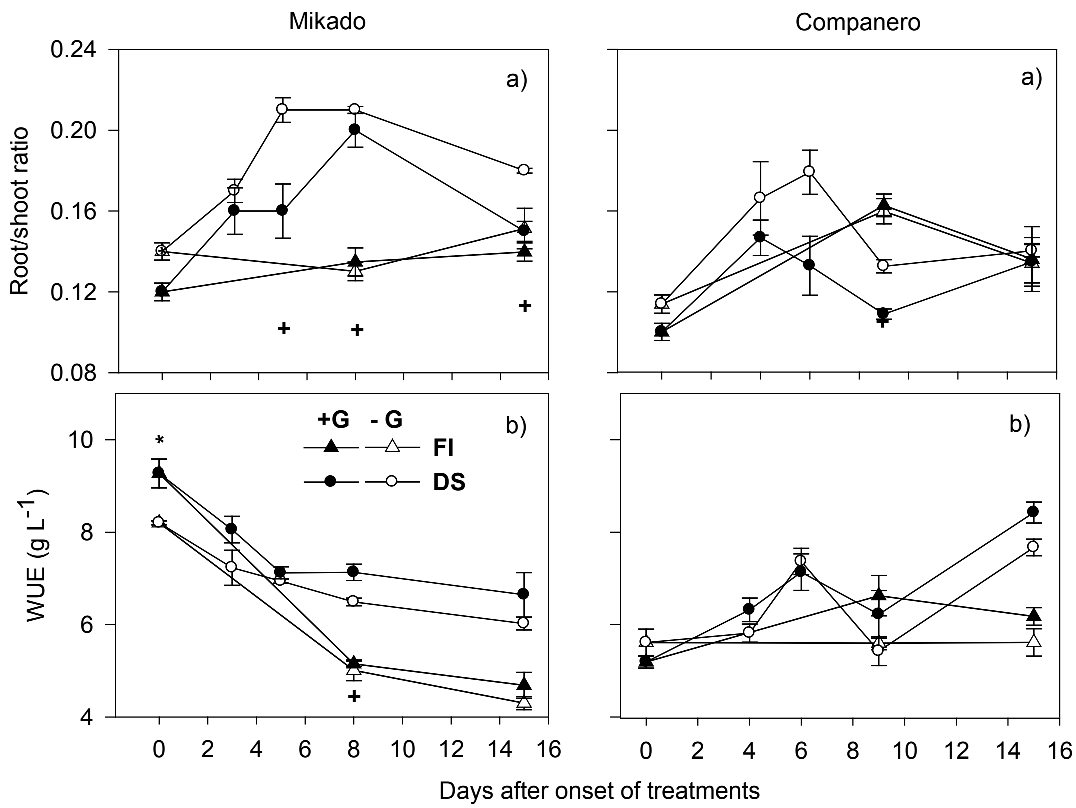


Fig. 3.12 Root-shoot ratio and water use efficiency (WUE) of two maize cultivars Mikado and Companero as influenced by Geohumus application under full irrigation and progressive drought

Note: root and shoot ratio (a), and water use efficiency (WUE) (b) of two cultivars (Mikado and Companero) over period of observation.FI: full irrigation; DS: drought spell; -G: sandy soil; and +G: sandy soil plus Geohumus Significant difference between control and Geohumus under FI (*) or DS (+) condition at alpha = 5%, n = 3. Error bars = standard deviation.

a) Root shoot ratio (RS)

In both cultivars root shoot ratio on control under drought spell was higher than on Geohumus; significant differences were found at onset, day 5, 8 and 15 (Mikado) and day 9 (Companero) (**Fig. 3.12 a**). There was no considerable difference in RS of both cultivars under FI between control and Geohumus.

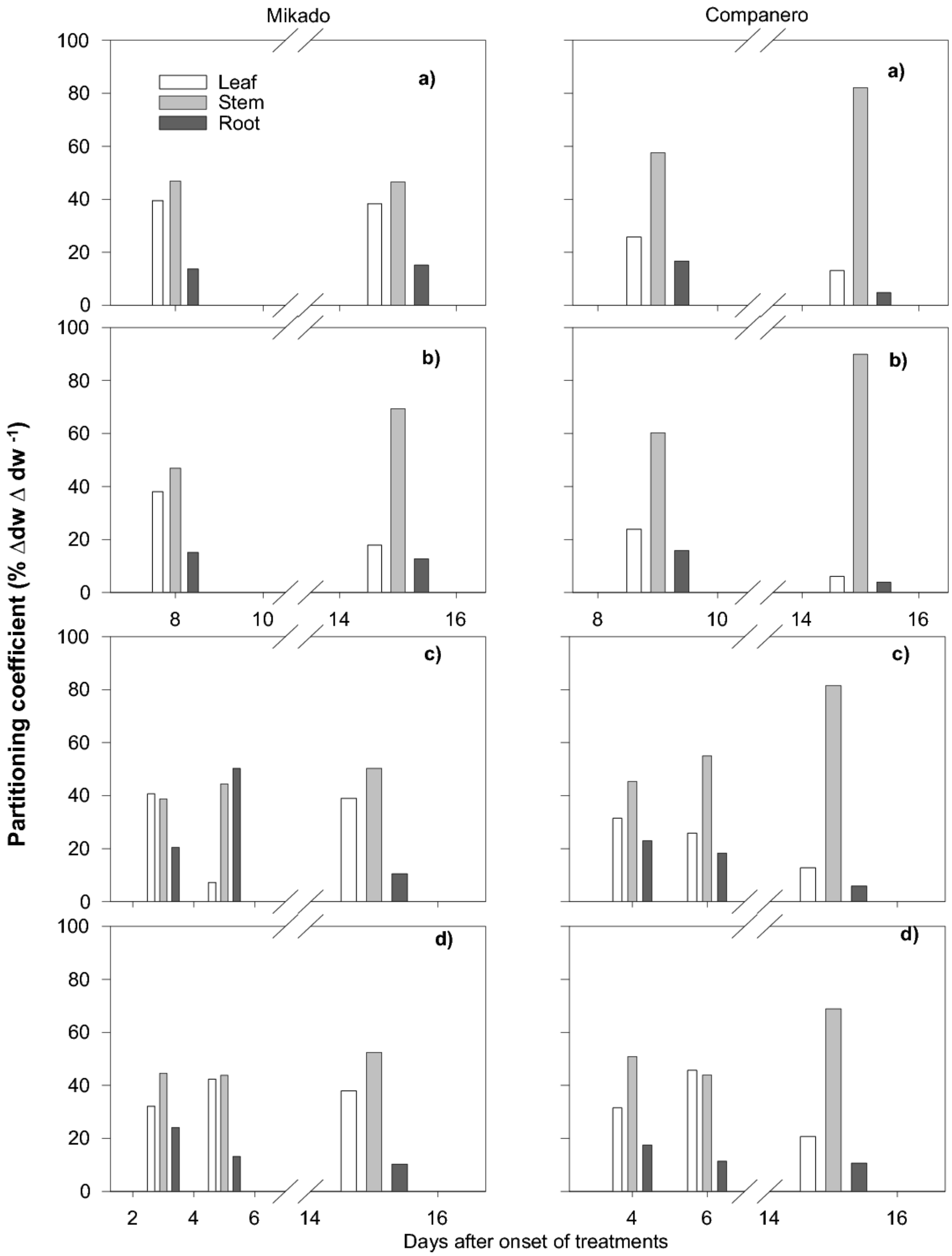


Fig. 3.13 Mean partitioning coefficients for leaves, stems and roots of two maize cultivars Mikado and Companero as influenced by Geohumus application under full irrigation and progressive drought

Note: full irrigation: without Geohumus (a) and with Geohumus (b); drought spell: without Geohumus (c) and with Geohumus (d).

b) Partitioning Coefficient

Under full irrigation, Mikado partitioning coefficient for stem on Geohumus at day 15 was higher than control; however, partitioning coefficient for leaf was reverse (**Fig. 3.13**). At day 5, considerable increase in Mikado partitioning coefficient for root on control and for leaf on Geohumus under drought spell was noted. Regarding Companero, partitioning coefficient for stem on control and partitioning coefficient for leaf on Geohumus at day 6 under drought spell remarkably increased comparing to previous period. No remarkable changes in partitioning coefficient for leaf, stem, and root of two cultivars day 15 on both control and Geohumus treatment under any water regime was observed.

3.2.4 Non-hydraulic signals: leaf, xylem ABA, and xylem pH

a) xylem pH (pH_{xylem})

Under any water conditions, no significant distinction in pH_{xylem} of Mikado and Companero between control and Geohumus treatment was found (**Fig. 3.14 a**). It seemed that Mikado pH_{xylem} under drought spell showed a decrease along with increasing dehydration and increase back after re-watering, but under full irrigation, it was stable. However, Companero pH_{xylem} under both drought as well as full irrigation illustrated linear increase during period of observation.

b) Leaf abscisic acid ($[ABA]_{leaf}$), xylem abscisic acid ($[ABA]_{xylem}$), and xylem pH (pH_{xylem})

Under prolonged drought, $[ABA]_{leaf}$ in both control and Geohumus showed the highest values at day 8 (Mikado) and 9 (Companero), but $[ABA]_{leaf}$ in Geohumus was remarkably higher than in control. Further, significant difference in Mikado $[ABA]_{leaf}$ at day 5 between Geohumus (0.16 µg DM^{-1}) and control (0.36 µg DM^{-1}) was also observed (**Fig. 3.14 b**). Under full irrigation, Companero $[ABA]_{leaf}$ in control was significantly higher than in Geohumus. The pattern of $[ABA]_{xylem}$ was very similar to $[ABA]_{leaf}$; significant difference in Mikado and Companero $[ABA]_{xylem}$ under drought spell between control and Geohumus was found at day 8 and 9 respectively (**Fig. 3.14 c**).

3.2.5 Plant water status

Root water potential, leaf water potential, and xylem osmotic potential of both Mikado and Companero on the control and Geohumus treatment showed similar pattern; they decreased along with increasing drought level under prolonged drought but quite stable under full irrigation (**Fig. 3.15 a, b, and d**). Significant differences in Mikado leaf water potential at day 3 and 8 and in Companero xylem osmotic potential at day 4 on Geohumus were significantly higher compared to the control.

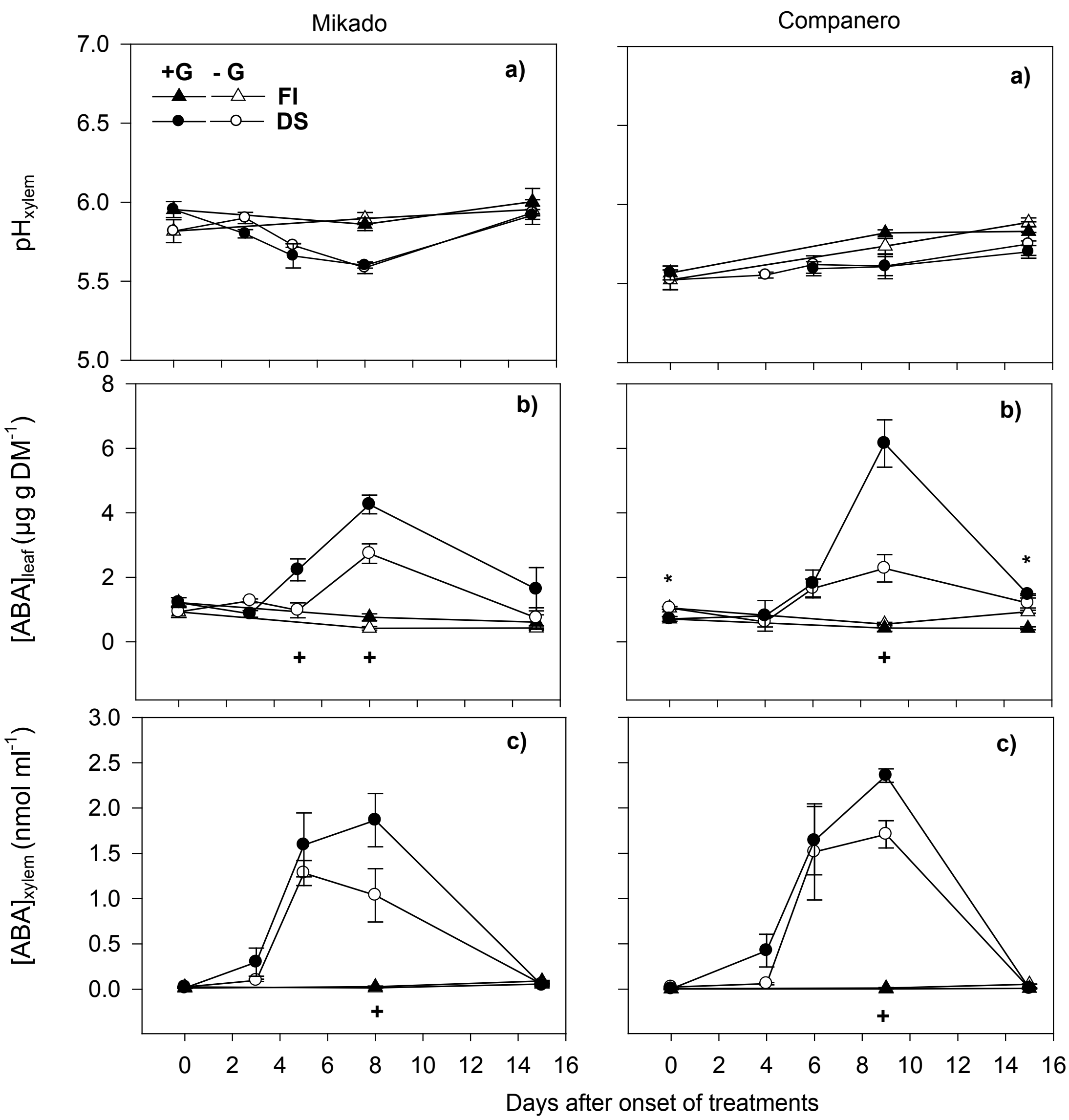


Fig. 3.14 pH$_{xylem}$ (a), [ABA]$_{leaf}$ (b), and [ABA]$_{xylem}$ (c) content of two maize cultivars Mikado and Companero as influenced by Geohumus application under full irrigation and progressive drought

Note: FI: full irrigation; DS: drought spell; -G: sandy soil; and +G: sandy soil plus Geohumus. Significant difference between control and Geohumus under FI (*) or DS (+) condition at alpha = 5%, n = 3. Error bars = standard deviation.

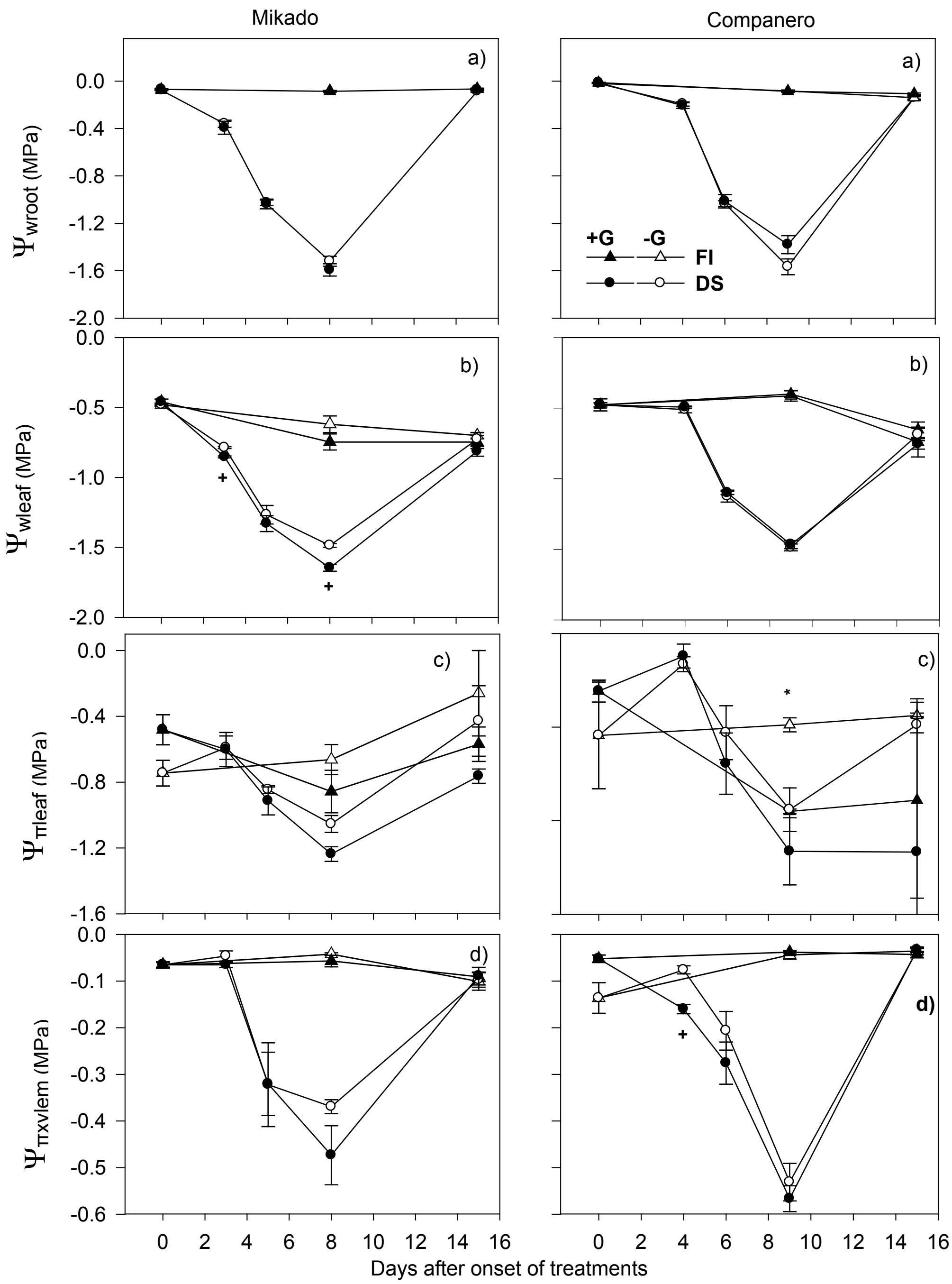


Fig. 3.15 Leaf (a) and root (b) water potential, leaf (c) and xylem (d) osmotic potential of two maize cultivars Mikado and Companero as influenced by Geohumus application under full irrigation and progressive drought

Note: Ψwroot: root water potential (a); Ψwleaf: leaf water potential (b); Ψπleaf: leaf osmotic potential (c), and Ψπxylem: xylem osmotic potential (d) of two cultivars over period of observation. FI: full irrigation; DS: drought spell; -G: sandy soil; and +G: sandy soil plus Geohumus. Significant difference between control and Geohumus under FI (*) or DS (+) condition at alpha = 5%, n = 3. Error bars = standard deviation..

3.2.6 *Leaf gas exchanges*

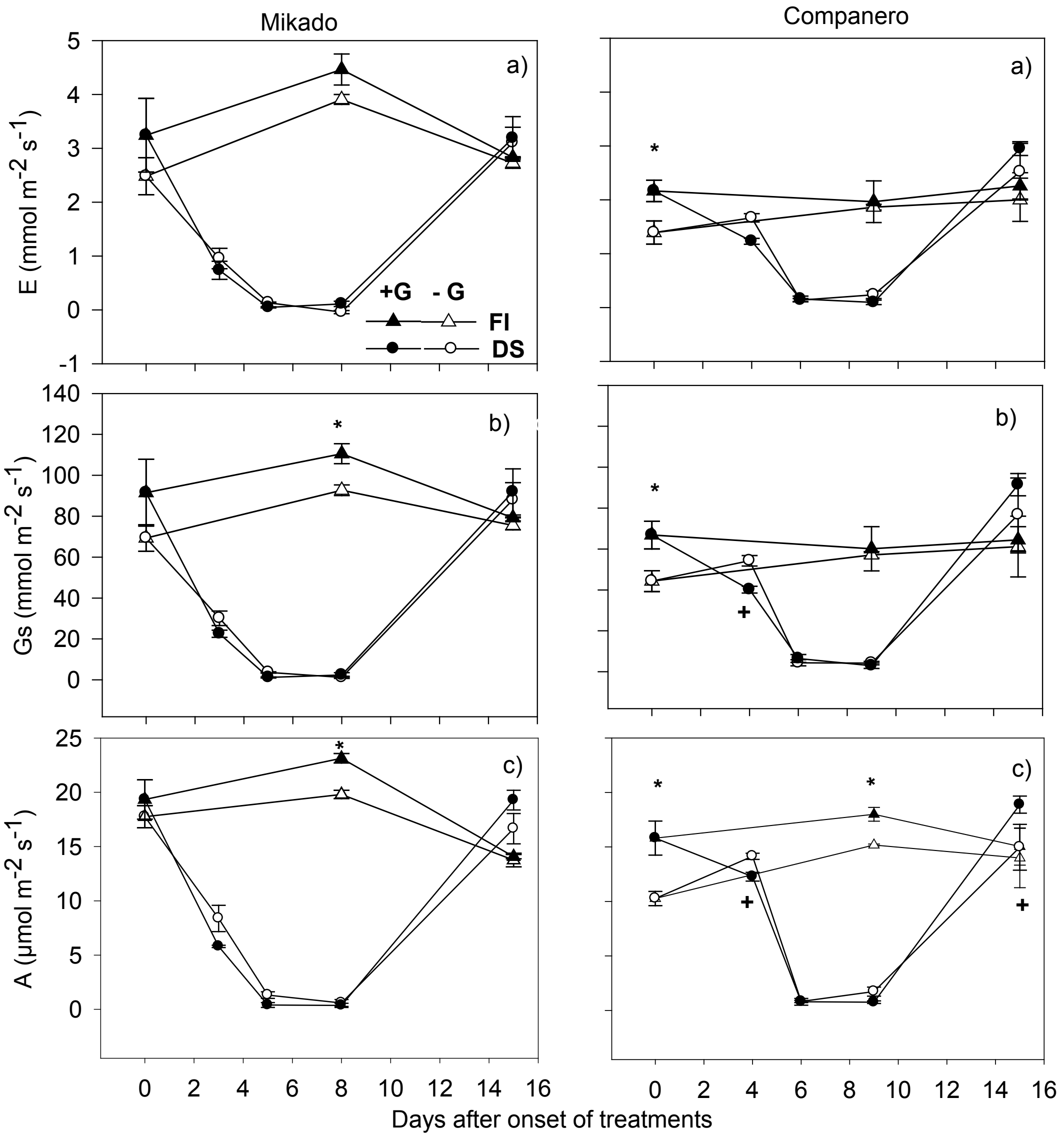


Fig. 3.16 Kinetics of transpiration (a), stomatal conductance (b) and assimilation rate (c) of two maize cultivars Mikado and Companero as influenced by Geohumus application under full irrigation and progressive drought

Note: FI: full irrigation; DS: drought spell; -G: sandy soil; and +G: sandy soil plus Geohumus. E: transpiration; Gs: stomatal conductance and A: assimilation rate. Significant difference between control and Geohumus under FI (*) or DS (+) condition at alpha = 5%, n = 3. Error bars = standard deviation.

Under full irrigation, Mikado and Companero gas exchange (transpiration, stomatal, and assimilate rate) on Geohumus were generally higher than the control (**Fig. 3.16**); significant difference in Companero gas exchange (on onset), assimilation rate (on day 8,

9) and Mikado stomatal conductance were found. Under drought spell, both Mikado and Companero assimilation rate with Geohumus on day 15 was slightly higher compared to the control. Similar to plant water status, gas exchange of both cultivars was likely affected by soil moisture content.

3.2.7 Hydrophilic polymer effects on water use efficiency (WUE)

Mikado and Companero WUE under FI and DS showed the same pattern in that WUE on Geohumus was generally superior to on control; however, significant difference in Mikado was found at onset and day 8 only. It is interesting that Mikado WUE on both control and Geohumus under FI and DS showed gradual decrease with increasing vegetable development while Companero WUE under DS was reversed (**Fig. 3.12 b**).

Summary of the drought spell experiment results:

- Green leaf area of Mikado and Companero on Geohumus treatments under full irrigation and drought spell tended to be higher than control. Leaf and shoot dry weight of both cultivars with Geohumus under full irrigation were higher compared to control, while under drought spell, they indicated no significant different between Geohumus and control, except in case of Mikado leaf, shoot weight at day 5.
- Total root dry weight of two cultivars on Geohumus under full irrigation seemed higher than control while it was reverse under drought spell. This pattern was also true for root length density in the last two layers but not the first two layers.
- Geohumus application under full irrigation and prolonged drought governed early Mikado root penetration to lower layers while Companero had no response to Geohumus at all.
- Under drought spell, root-shoot ratio of Mikado and Companero on control was higher than on Geohumus
- Under drought spell, at day 5, considerable increase Mikado partitioning coefficient for roots on control but for leaf on Geohumus while at day 6, Companero partitioning coefficient for stem on control and for leaf on Geohumus; under full irrigation, at day 15, increase of Mikado partitioning coefficient for

stem but decrease of that for leaf on Geohumus while no significant change in partitioning coefficient between leaf, stem, and root on control.

- Geohumus application showed no considerable effect on pH_{xylem} of both Mikado and Companero under full irrigation and drought spell as well.
- $[ABA]_{leaf}$ and $[ABA]_{xylem}$ of both cultivars on Geohumus under drought spell showed significant difference at the most severe drought point.
- Mikado and Companero root water potential, leaf water potential, and xylem osmotic potential on both control and Geohumus treatment under drought spell showed positive relationship with soil moisture. Geohumus application resulted in lower root water potential, leaf water potential, and xylem osmotic potential.
- Gas exchange (E, Gs, and A) of two cultivars on Geohumus treatment under full irrigation tented higher than control and A of these cultivars subjected to drought on Geohumus was also higher on day 15.
- WUE of both cultivars on Geohumus under both full irrigation and drought seemed higher than control.

3.3 Maize response to Geohumus applied in a split root system with different water regimes and soil

3.3.1 pH$_{xylem}$, [ABA]$_{leaf}$, and [ABA]$_{xylem}$

*** pH$_{xylem}$**

Mikado pH$_{xylem}$ decreased with increasing water deficit while Companero pH$_{xylem}$ showed no trend throughout water treatments (**Table 3.8**). Mikado grown in sandy soil had lower pH$_{xylem}$ compared to compost soil. Geohumus application to sandy soil increased Mikado pH$_{xylem}$ only at deficit irrigation. In contrast to Mikado, Companero grown in compost soil resulted in lower pH$_{xylem}$ compared to plant grown in sandy soil. Geohumus had no effect on pH$_{xylem}$ under both soil types.

Table 3.8 Effects of Geohumus, soil types and water regime on pH$_{xylem}$ of two cultivars

Treatment	Cultivars					
	Mikado			Companero		
	FI$_{SRS}$	PRD	DI	FI$_{SRS}$	PRD	DI
Sandy soil (SS)	5.52a C	5.47b B	5.33b C	5.86b A	6.13a A	5.91b A
SS + Geo	5.68a BC	5.46b B	5.47b B	5.91a A	5.94a A	5.89a A
Compost (C)	5.82a AB	5.78a A	5.59b A	5.46a B	5.37a B	5.39a B
C + Geo	5.87a A	5.76ab A	5.68b A	5.37a B	5.40a B	5.41a B

Note: SS+Geo: sandy soil plus Geohumus; C+Geo: Compost plus Geohumus; FI$_{SRS}$: Full irrigation on Split root system; PRD: partial root drying; DI: irrigation deficit. Capital and normal letter indicate for comparing between columns and rows in the experiment. Differences in letter significantly denote between treatments at alpha 5%; n = 5-11.

*** Leaf ABA concentration [ABA]$_{leaf}$**

Soil types without Geohumus had more effects on [ABA]$_{leaf}$ of both cultivars compared to the water treatments (**Table 3.9**). Mikado differently responded to soil types in [ABA]$_{leaf}$ e.g. Geohumus application to sandy soil increased [ABA]$_{leaf}$ of Mikado up to 2-3 times higher under comparable conditions; however, the pattern was opposite when Geohumus applied to compost. Further, Geohumus application to sandy soil and compost led to change in the pattern of Mikado [ABA]$_{leaf}$ throughout the water treatments but not that of Companero [ABA]$_{leaf}$.

*** Xylem ABA concentration ([ABA]$_{xylem}$)**

Table 3.9 Effects of Geohumus, soil types and water regime on leaf ABA (µg g^{-1} DM) of two cultivars

Treatment	Cultivar					
	Mikado			Companero		
	FI$_{SRS}$	**PRD**	**DI**	**FI$_{SRS}$**	**PRD**	**DI**
Sandy soil (SS)	9.79a C	12.10a C	13.23a C	2.49a A	4.16a A	5.72a A
SS + Geo	20.69b AB	28.58b A	38.13a A	1.82a A	3.7a AB	4.24a A
Compost (C)	23.42ab A	19.59b B	27.84a AB	2.00a A	2.3a B	2.59a A
C + Geo	16.11a B	17.52a BC	20.29a BC	2.78a A	2.9a AB	4.77a A

Note: SS+Geo: sandy soil plus Geohumus; C+Geo: Compost plus Geohumus; FI$_{SRS}$: Full irrigation on Split root system; PRD: partial root drying; DI: deficit irrigation. Capital and normal letters indicate for comparing between columns and rows in the experiment. Differences in letter significantly denote between treatments at alpha 5%; n = 5-11.

Table 3.10 Effects of Geohumus, soil types and water regime on [ABA]$_{xylem}$ (nmol ml^{-1}) of two cultivars

Treatment	Cultivar					
	Mikado			Companero		
	FI$_{SRS}$	**PRD**	**DI**	**FI$_{SRS}$**	**PRD**	**DI**
Sandy soil (SS)	0.45b AB	0.46b A	1.99a B	0.30c C	0.88b AB	2.76a A
SS + Geo	0.51b AB	1.03b A	4.87a A	0.19b C	1.92a A	2.28a A
Compost (C)	0.32b B	0.97b A	1.67a B	0.56a B	0.69a B	1.54a A
C + Geo	0.76b A	1.04b A	2.29a B	0.83a A	1.95a A	2.06a A

Note: SS+Geo: sandy soil plus Geohumus; C+Geo: Compost plus Geohumus; FI$_{SRS}$: Full irrigation on Split root system; PRD: partial root drying; DI: irrigation deficit. Capital and normal letter indicate for comparing between columns and rows in the experiment. Differences in letter significantly denote between treatments at alpha 5%; n = 5-11.

Unlike to [ABA]$_{leaf}$, the water treatment had more effect on Mikado [ABA]$_{xylem}$ compared to soil types (**Table 3.10**). Geohumus application to sandy soil and compost increased Mikado [ABA]$_{xylem.}$ It is notable is that Mikado [ABA]$_{xylem}$ on both sandy soil and compost with or without Geohumus showed the same pattern in that [ABA]$_{xylem}$ under deficit irrigation had highest concentration and significantly differed compared to under full irrigation and partial root drying as well. Referring to Companero, the water treatment affected on Companero $[ABA]_{xylem}$ on only sandy soil with or without Geohumus. Similar to Mikado, Companero grown in compost with Geohumus under full irrigation and partial root drying had significantly higher $[ABA]_{xylem}$ than control under comparable conditions.

3.3.2 Plant water status

*** Leaf water potential (Ψ_{wleaf}) on SRS experiments**

Mikado grown on compost under partial root drying and deficit irrigation had significantly lower Ψ_{wleaf} than grown on sandy soil; this was reverse for Companero Ψ_{wleaf}. Geohumus application resulted in increase of Mikado Ψ_{wleaf} in sandy soil under partial root drying and deficit condition and Companero Ψ_{wleaf} in compost under three water treatments. Mikado Ψ_{wleaf} on both sandy soil and compost with or without Geohumus decreased with decreasing soil moisture while that is true for Companero Ψ_{wleaf} on sandy soil with or without Geohumus.

Table 3.11 Effects of Geohumus, soil types and water regime on leaf water potential (MPa) of two cultivars

Treatment	Cultivar					
	Mikado			Companero		
	FI_{SRS}	PRD	DI	FI_{SRS}	PRD	DI
Sandy soil (SS)	-0.55b A	-0.56ab B	-0.75a B	-0.26c B	-0.88b AB	-1.31a A
SS + Geo	-0.51b A	-0.86a A	-0.97a A	-0.21 b B	-1.14a A	-1.22a A
Compost (C)	-0.42c A	-0.78b A	-1.05a A	-0.37 a B	-0.39 a C	-0.43a C
C + Geo	-0.65b A	-0.71b AB	-0.89a AB	-0.56a A	-0.63a BC	-0.64a B

Note: SS+Geo: sandy soil plus Geohumus; C+Geo: Compost plus Geohumus; FI_{SRS}: Full irrigation on Split root system; PRD: partial root drying; DI: irrigation deficit. Capital and normal letter indicate for comparing between columns and rows in the experiment. Differences in letter significantly denote between treatments at alpha 5%; n = 5-11.

*** Root water potential (Ψ_{wroot}) on SRS experiments**

Similar to $\Psi_{wleaf,}$ Mikado grown on sandy soil had Ψ_{wroot} lower than that grown on compost and Geohumus application to sandy soil under partial root drying and deficit irrigation reduced Mikado Ψ_{wroot} compared to control under the same condition (**Table**

3.12). However, Geohumus application to two soil types had no effect on Companero $\Psi_{wroot.}$ Throughout the water treatments, Mikado and Companero grown in soil types with Geohumus showed change in the pattern of Ψ_{wroot}.

Table 3.12 Effects of Geohumus, soil types and water regime on Ψ_{wroot} (MPa) of two cultivars

Treatment	**Cultivar**					
	Mikado			**Companero**		
	FI_{SRS}	**PRD**	**DI**	**FI_{SRS}**	**PRD**	**DI**
Sandy soil (SS)	-0.38a A	-0.43a B	-0.55a B	-0.023b C	-0.66a AB	-1.00a A
SS + Geo	-0.33c A	-0.65b A	-0.80a A	-0.04b BC	-0.71a A	-0.98a A
Compost (C)	-0.27c A	-0.56b AB	-0.80a A	-0.16b A	-0.30a B	-0.40a B
C + Geo	-0.34b A	-0.45b B	-0.71a AB	-0.29a A	-0.32a B	-0.41a B

Note: SS+Geo: sandy soil plus Geohumus; C+Geo: Compost plus Geohumus; FI_{SRS}: Full irrigation on Split root system; PRD: partial root drying; DI: irrigation deficit. Capital and normal letter indicate for comparing between columns and rows in the experiment. Differences in letter significantly denote between treatments at alpha 5%; n = 5-11.

Table 3.13 Effects of Geohumus, soil types and water regime on $\Psi_{\pi leaf}$ (MPa) of two cultivars

Treatment	**Cultivar**					
	Mikado			**Companero**		
	FI_{SRS}	**PRD**	**DI**	**FI_{SRS}**	**PRD**	**DI**
Sandy soil (SS)	-0.73b A	-0.67b A	-1.11a A	-0.81c A	-1.04b A	-1.23a A
SS + Geo	-0.67a A	-0.64a A	-0.80a A	-0.90b A	-1.17a A	-1.13a A
Compost (C)	-0.66b A	- 0.77ab A	-0.97a A	-0.28b B	-0.31b B	-0.39a B
C + Geo	-0.57a A	-0.63a A	-0.89a A	-0.28a B	-0.34a B	- 0. 36a B

Note: SS+Geo: sandy soil plus Geohumus; C+Geo: Compost plus Geohumus; FI_{SRS}: Full irrigation on Split root system; PRD: partial root drying; DI: irrigation deficit. Capital and normal letter indicate for comparing between columns and rows in the experiment. Differences in letter significantly denote between treatments at alpha 5%; n = 5-11.

*** Leaf osmotic potential ($\Psi_{\pi leaf}$) on SRS experiments**

Both water treatments and soil types had effect on Companero $\Psi_{\pi leaf}$ while the water treatment affected on Mikado $\Psi_{\pi leaf}$ in compost only (Table 3.13). It is notable that Geohumus application to sandy soil and compost showed no change in $\Psi_{\pi leaf}$ of two cultivars under comparable water conditions. However, Geohumus changed the pattern of

Mikado and Companero $\Psi_{\pi leaf}$ grown on both sandy soil and compost throughout three water supply levels.

*** xylem osmotic potential ($\Psi_{\pi xylem}$)**

Companero $\Psi_{\pi xylem}$ responded stronger to soil types than the water treatment while Mikado $\Psi_{\pi xylem}$ was absolutely reversed (**Table 3.14**). Geohumus application to sandy soil resulted in decrease of Mikado $\Psi_{\pi xylem}$ (under partial root drying and deficit irrigation) and Companero $\Psi_{\pi xylem}$ (under deficit irrigation); besides, it also caused change in the pattern of Mikado $\Psi_{\pi xylem}$ grown on soil types throughout water treatments.

Table 3.14 Effects of Geohumus, soil types and water regime on sap osmotic potential (MPa) of two cultivars

Treatment	Cultivar					
	Mikado			Companero		
	FI_{SRS}	**PRD**	**DI**	**FI_{SRS}**	**PRD**	**DI**
Sandy soil (SS)	-0.080a A	-0.070a B	-0.110a B	-0.120a A	-0.14a AB	-0.130a B
SS + Geo	-0.090b A	-0.140ab A	-0.160a A	-0.160a A	-0.18a A	-0.290a A
Compost (C)	- 0.077b A	-0.106b AB	-0.144a AB	-0.056a B	-0.052a B	-0.054a C
C + Geo	-0.088 c A	-0.093bc AB	-0.143a AB	-0.063a B	-0.066a B	-0.067a C

Note: SS+Geo: sandy soil plus Geohumus; C+Geo: Compost plus Geohumus; FI_{SRS}: Full irrigation on Split root system; PRD: partial root drying; DI: irrigation deficit. Capital and normal letter indicate for comparing between columns and rows in the experiment. Differences in letter significantly denote between treatments at alpha 5%; n = 5-11.

3.3.3 Stomatal conductance (Gs)

The water treatment had more effect on Mikado Gs than soil types while Companero Gs was affected by not only water treatments but also soil types (**Table 3.15**). Geohumus application to both sandy soil and compost had no change in Gs of two cultivars under comparable water treatment, except to sandy soil under partial root drying (Mikado) and under full irrigation (Companero) and to compost under deficit irrigation (Companero). Partial root drying resulted in significant decrease in Gs of two cultivars grown on two soil types with or without Geohumus compared to full irrigation but seemed likely higher than deficit irrigation.

Table 3.15 Effects of Geohumus, soil types and water regime on predicted Gs (mmol m^{-2} s^{-1}) of two cultivars

Treatment	Cultivar					
	Mikado			Companero		
	FI_{SRS}	**PRD**	**DI**	**FI_{SRS}**	**PRD**	**DI**
Sandy soil (SS)	28.03a A	24.68ab A	18.84b A	87.23 a A	22.36 b B	10.42 c C
SS + Geo	29.74a A	15.87b B	11.47b A	73.06 a B	16.77 b B	10.30 b C
Compost (C)	36.12a A	20.36 b AB	12.03c A	50.67 a C	34.93 b A	33.39 b A
C + Geo	33.26a A	25.10 b A	15.55c A	45.69 a C	25.76 b AB	24.08 b B

Note: SS+Geo: sandy soil plus Geohumus; C+Geo: Compost plus Geohumus; FI_{SRS}: Full irrigation on Split root system; PRD: partial root drying; DI: irrigation deficit. Capital and normal letter indicate for comparing between columns and rows in the experiment. Differences in letter significantly denote between treatments at alpha 5%; n = 5-11.

3.3.4 Green leaf area

Table 3.16 Effects of Geohumus, soil types and water regime on green leaf areas (cm^2) of two cultivars.

Treatments	Cultivars					
	Mikado			Companero		
	FI	**PRD**	**ID**	**FI**	**PRD**	**ID**
Sandy soil (SS)	105a B	81a C	90a B	454a B	471a B	454a B
SS + Geo	194a A	205a B	221a A	613a A	561a A	629a A
Compost (C)	237a A	279a A	272a A	340a C	288 a D	307a C
C + Geo	227a A	190a B	213a A	388a BC	368 a C	372a C

Note: SS+Geo: sandy soil plus Geohumus; C+Geo: Compost plus Geohumus; FI_{SRS}: Full irrigation on Split root system; PRD: partial root drying; DI: deficit irrigation. Capital and normal letters indicate for comparing between columns and rows in the experiment. Differences in letter significantly denote between treatments at alpha 5%; n = 5-11.

Leaf area of two cultivars grown on compost was superior to on sandy soil (**Table 3.16**). Geohumus application led to greater leaf area of Mikado (on sandy soil) and Companero (on two soil types) but less leaf area of Mikado (on compost). There were no effect of the water treatments on leaf area of both cultivars grown sandy soil and compost with or without Geohumus.

3.3.5 Summary of results from split root system experiments

a) Genotypic response to water treatments

- Mikado pH_{xylem} showed positive relationship with soil moisture but Companero pH_{xylem} had no response to the water treatment
- $[ABA]_{leaf}$ of both cultivars on two soil types with or without Geohumus did not respond to the water treatment, except Mikado $[ABA]_{leaf}$ on sandy soil with Geohumus and Compost without Geohumus increased with increasing water deficit.
- $[ABA]_{xylem}$ of Mikado on sandy soil and compost and Companero on sandy soil showed negative correlation with soil moisture
- Ψ_{wleaf}, Ψ_{wroot}, $\Psi_{\pi leaf}$, $\Psi_{\pi xylem}$, and Gs of Mikado on two soil types with or without Geohumus generally reduced with decreasing soil water content. However, these parameters were not significantly affected by the water treatments.

In short, the values of non-hydraulic signals, plant water status, and stomatal conductance of two cultivars under partial root drying were inside full irrigation and deficit irrigation range.

b) Genotypic response to soil types (without Geohumus)

Under comparable water treatment, genotypic response to soil types shown in detail following:

- Mikado grown on compost resulted in higher pH_{xylem} and $[ABA]_{leaf}$ than on sandy soil; but $[ABA]_{xylem}$, $\Psi_{\pi leaf}$, $\Psi_{\pi xylem}$, and Gs had no response to soil types while Mikado Ψ_{wleaf}, Ψ_{wroot} on compost under partial and deficit irrigation were significantly lower than sandy soil.
- Companero grown on compost showed non-hydraulic signals, plant water status, stomatal conductance superior to grown on sandy soil, except pH_{xylem}.
- Mikado and Companero grown on compost had greater leaf area than on sandy soil

c) Genotypic response to Geohumus

- Geohumus application to sandy soil led to decrease of Mikado pH_{xylem} only.

- Companero $[ABA]_{leaf}$ had no response to sandy soil and Compost with Geohumus at all while Mikado $[ABA]_{leaf}$ showed opposite responses to Geohumus according to soil types (increase on sandy soil but decrease on compost)
- $[ABA]_{xylem}$ of both cultivars on Geohumus treatments seemed generally higher than on controls
- Geohumus application to sandy soil led to reduction of Mikado Ψ_{wleaf}, Ψ_{wroot} and $\Psi_{\pi xylem}$ under partial root drying and deficit irrigation while compost with Geohumus showed negative effect on Companero Ψ_{wleaf} (on compost under three water treatments) and Companero $\Psi_{\pi xylem}$ (on sandy soil under deficit irrigation)
- Gs of two cultivars were generally reduced by Geohumus application, except Mikado Gs on compost.
- Geohumus application to two soil types led to greater leaf area of Companero; this trend was true for Mikado grown on sandy soil with Geohumus but opposite for Mikado grown on compost.

4 DISCUSSION

This section will discuss the underlying hypotheses of this research by addressing the following questions: do selected abiotic factors (temperature, soaking time, salt concentration and type, incorporation depth) affect on Geohumus water holding capacity and could its water holding capacity be restored? Does Geohumus application affect the morphological and physiological responses of two cultivars under conditions of prolonged water deficit? Does Geohumus in combination with soil type affect drought induced root-shoot communication?

4.1 Impact of selected abiotic factors on Geohumus water holding capacity (WHC) and restorability

4.1.1 Temperature

Water absorption of hydrophilic polymers in response to ambient temperatures depends on their properties; in fact, hydrogen bonds in polymer chains become weaker with increasing temperatures (Andry, Yamamoto et al. 2009). The results presented here, show that there was a sharp increase in WHC of Geohumus over a temperature range of 10-50^{o}C (**Fig. 3.1**). These results are in agreement with earlier studies that were conducted on carboxylmethylcellulose, with temperature range from 15-35^{o}C (Suo, Qian et al. 2007; Andry, Yamamoto et al. 2009) . When temperature was lower than 50^{o}C, the molecular chains of polymers were not broken, which would reduce the water absorption capacity of hydrophilic polymers. It should be noted that there was an interaction between temperature and the presence of ions in solution in which Geohumus was immersed. The WHC of Geohumus in distilled water and 10% nutrient solution strongly increased when temperature was above 10^{o}C, whereas in 100% nutrient solution the WHC very slightly increased within a range of 10-50^{o}C. According to this result, Geohumus possesses hydrogen bonds that could increasingly absorb water within the temperature range of 10-50^{o}C. In the present study, WHC of Geohumus decreased with increasing ion concentration at each temperature level, implying that, rather than temperature, chemical factors play an important role in determining WHC of Geohumus. It can be concluded that the application of Geohumus in relatively cold and/or salty environments will reduce WHC of the polymer and thereby its potential resources to provide plant available water.

4.1.2 Immersion duration

A logical assumption is that the longer a polymer is immersed in nutrient solution, the more ions it can absorb, resulting in reduced WHC. To clarify this deduction, Geohumus was used to evaluate the effect of time courses and soil water content in two media, on the WHC of Geohumus.

In solutions, significant differences in WHC of Geohumus were observed between two media (nutrient solution and de-ionized water) (**Fig. 3.2***)*. The peak WHC of Geohumus occurred after 4 hours, and decreased from 6 to 12 hours; WHC was fairly constant over the next period. On the contrary, there was a close correlation between WHC of Geohumus and time immersed in de-ionized water. This difference can be explained by the concentration of certain elements in nutrient solutions, and the changed electric conductivity, as compared to de-ionized water. Ions in solution replace or remove water from vacuoles (James and Richards 1986) or impair water absorption of polymer chains (Martin, Ruter et al. 1993), thereby reducing the ability of Geohumus to absorb water. According to the results shown in **Fig. 3.2**, it took about 12 hours to complete this process. On the contrary, WHC of Geohumus immersed in de-ionized water showed a dramatic increase during the trial, which may be explained by the slow diffusion of ions from Geohumus to de-ionized water following a concentration gradient, which led to an increased WHC, as Geohumus contains low concentrations of potassium, chloride, and nitrate.

4.1.3 Salts

a) Salt concentration

The most prominent issues mentioned in previous reports are the negative impact of salt content on WHC of polymers. In this research, a series of trials were conducted in order to compare the effect of various salt or electrical conductivity (EC) levels, aiming to estimate their impact on Geohumus WHC.

Significant differences in WHC of Geohumus immersed in tap water, nutrient solution, soil extract, nutrient plus soil extract, compost extract and compound of salts were observed with respect to salt content or EC. Geohumus immersed in solutions with high EC values expressed low WHC (**Fig. 3.1, 3.2, 3.3, 3.4, 3.5** and **Table 3.1, 3.3, 3.4**). Also, increasing concentrations of nutrients in solution led to a decline in WHC of Geohumus **(Fig. 3.4).** Although the trials were conducted separately at different times, results showed that there was a negative correlation between WHC of Geohumus, EC value and salt concentration (**Table 3.1**). These results were in agreement with many previous reports on similar products (Johnson 1984; AI-Darby, Mustafa et al. 1990; Smith and Harrison 1991). According to **Table 5.3**, a negative relationship between concentrations of most compounds and WHC of Geohumus was also observed.

WHC of Geohumus was very low in solutions such as tap water, nutrient solution, compost extract, soil extract plus nutrient solution and soil extract compared to distilled water (**Fig. 3.3**). Among solutions used, distilled water had lowest salt concentration, following tap water could be feasible source for irrigation with low salt concentration compared to the rest (**Table 2.1**), resulting in higher WHC. As these solutions contain certain ionic elements, which create ionic bridges between carboxyl-groups inside the matrix of the gel, thereby decreasing hydration of the gel by impairing electrical repulsion of aligned co-polymer chains (Martin, Ruter et al. 1993). Replacing and removing water by multivalent cations at polarized sites within hydrogels in solution leads to impeded water absorption of polymers (James and Richards 1986). The results show that soil property and water quality are two potential factors affecting WHC of Geohumus. Therefore, the consideration of soil properties prior to the application of polymers is essential. In addition, fertilizer application with the aim of improving soil quality needs to be considered, as the application of fertilizers could contribute to an increased soil EC, which may in turn lead to reduced hydration of Geohumus (**Fig. 3.4**). It may therefore be concluded the regular application of nutrient solution significantly improved the WHC of Geohumus compared to a single, base application of fertilizers. The ideal solution is that the application of fertilizers be divided into as many applications as possible in order to obtain advantages not only regarding the water absorption of Geohumus, but also plant nutrient uptake. In practice, rainwater could be considered as a source that could affect the WHC of polymers because it also contains certain salt concentration and specific ions (Root, Jones et al. 2004).

b) Valance types

*** Within group of monovalent ions**

Has been shown earlier, that a polymers' hydration performance may depend on salt concentration (Johnson 1984; AI-Darby, Mustafa et al. 1990; Dorraji, Golchin et al. 2010) and ion type (Johnson 1984; Smith and Harrison 1991; Green, Foster et al. 2004). In this section, ionic factors and salt compounds influencing water absorption of Geohumus are considered in detail.

Table 3.1 illustrated that there is no differences in WHC of Geohumus in two different solutions containing KNO_3 and NH_4NO_3 in the range of 0.01-0.2 M. This shows that K^+ and NH_4^+ have a similar capability of impeding water absorption of Geohumus by replacing and removing water molecules in the chains of Geohumus. In another pairing**,** K^+ hindered water absorption stronger than Na^+ in the range of 0.04-0.2 M. However, the difference in ionic strength of these ions was not large enough to create significant differences when salt concentrations ranged from 0.01 to 0.03M. This implies that the estimation of WHC of polymers in solutions should consider not only the property of ions but also the range of salt concentration. In this case, the low salt concentration masked the power of K^+ in impeding the water absorption of Geohumus.

*** Comparing between groups with monovalent and multivalent ions**

When diluted, K^+ in K_2SO_4 solution should have a higher K concentration than in KCl because the osmolality of the potassium sulphate solution is about 100% higher than that of potassium chloride. However, in the range of 0.05- 0.2M, the hydration of Geohumus in KCl was lower than in K_2SO_4 (**Table 3.1**). Geohumus showed the complete opposite behavior when immersed in solutions ranging 0.03-0.01M. In this case, it is not possible to state that Cl^- was stronger than the SO_4^{2-} ion, as the difference was not only the presence of either Cl^- or SO_4^{2-}, but also the concentration of K^+. A possible explanation for this phenomenon is that at concentrations of 0.05- 0.2M, counteraction would take place between K^+ and SO_4^{2-} in K_2SO_4 solution, leading to better Geohumus expansion compared to KCl, although SO_4^{2-} is much stronger than Cl^- in limiting water absorption (Johnson 1984; Green, Foster et al. 2004) and the presence of SO_4^{2-} may lower the concentration of ions (0.03-0.01M). Another match, K_2SO_4 solution did hinder Geohumus expansion to a much lower extend as compared to $MgSO_4$. This means that higher osmolality does not play a more important role in impeding WHC of Geohumus than valence type. Multivalent ions more strongly than monovalent ions impeded WHC of polymers (Johnson 1984; Green, Foster et al. 2004). Therefore, the negative impact of monovalent ions was ranked in a decreasing order: $Na^+ > NH_4^+ \approx K^+$.

*** Within groups of multivalent ions**

Comparisons of the sulphates of iron and magnesium showed that the WHC of Geohumus in $MgSO_4$ solution was significantly higher than in $FeSO_4$ solution at all concentrations that were tested. We can therefore conclude that Fe^{2+} is significantly more effective than Mg^{2+} in hindering WHC of Geohumus in soluble conditions (**Table 3.1**). A similar trend was observed when Geohumus was immersed in $FeSO_4$ and $Al_2O_{12}S_3$ with $FeSO_4$ showing stronger negative effect on WHC of Geohumus than $Al_2O_{12}S_{31}$ in almost all concentrations except 0.01M. This result is in complete agreement with previous report on polyacrylamide gels (Soma and Soma 1989; Martin, Ruter et al. 1993). The stronger hindrance of water absorption the ions are, the more density it could occupy in polymer chains (Chen, Zommorodi et al. 2003). In short, the strength of cations hindering water absorption of Geohumus in decreasing order is as follows: $Fe^{2+}>Al^{3+}> Mg^{2+}$.

*** Counteraction among ions in solution**

To estimate the phenomenon of counteraction of ions in solution, five compounds, including NaCl, KCl, K_2SO_4, $MgSO_4$ and $FeSO_4$, were mixed together. The concentration of each compound in mixed solution was decreased 5 times with respect to its own concentration. So the concentration in mixed solution at 0.2 is equal to its former solution at 0.04M. The result in **Table 3.1** showed that the Fe-compound was the strongest in impeding the hydration of Geohumus. If it is assumed that 100% $FeSO_4$ could be absorbed by Geohumus, the WHC value of Geohumus in mixed solution at 0.2M should be similar to $FeSO_4$ solution at 0.04M. However, WHC value of Geohumus in mixed solution at 0.2M was doubled compared to pure $FeSO_4$ solution. This means that there was a counteraction or competition among ions in mixed solution, leading to reduced absorption of iron in mixed solution. The value of WHC in mixed solution at 0.2M was most similar to $FeSO_4$ solution at 0.04, compared to others at the same concentration. This can be interpreted as iron being the most dominant ion in hindering water absorption of Geohumus relative to the other ions. Similar results were found when mixed solutions at 0.1 or 0.05M were compared to other solutions at 0.02 or 0.01M.

*** Comparison between multivalent and monovalent ions**

According to figures shown in **Table 3.1**, **Fig. 3.5, and Table 7.1**, it is clear that compounds containing monovalent ions such as KNO_3, NH_4NO_3, NaCl, KCl and K_2SO_4 were remarkably weak in hindering the hydration of Geohumus in solutions, compared to compounds with multivalent ions i.e., $MgSO_4$, $FeSO_4$ and $Al_2O_{12}S_3$. Significant differences between types of monovalent and multivalent ions may be attributed to the presence of oxygen atoms in Geohumus polymer chains that attract multivalent ions such as Ca^{2+} more than monovalent elements such as Na^+ leading to higher concentrations of Ca^{2+} compared to Na^+ in polymers (Chen, Zommorodi et al. 2003).

4.1.4 Incorporation depth

The effect of soil density on WHC of Geohumus must be considered. Soil density may affect not only root growth but also the expansion of any material in the soil, such as Geohumus. Soil density of the topsoil of pots after one crop of maize was significantly lower than at lower layers (**Table 3.5**), but there was no significant difference in WHC of used Geohumus among the four soil layers (**Fig. 3.6**), implying that soil density did not affect WHC of used Geohumus. We can also note that WHC of Geohumus in all soil layers was low making it difficult to conclude that there was no impact of soil density on WHC, as used Geohumus contributed two factors, namely soil density and salt, to soil. Our trial illustrated that the EC value increased gradually from the top to bottom layer, while the WHC of Geohumus was the opposite; negative effect of EC was demonstrated by many previous reports (Johnson 1984; AI-Darby, Mustafa et al. 1990; Martin, Ruter et al. 1993; Dorraji, Golchin et al. 2010). There were significant differences in WHC between control and treatment at the last two layers (**Table 3.3**); this considerable difference was attributed to nutrient application and confirmed in **Section 3.1.1, 3.1.2,** and **3.1.3** in this research and by earlier results (Martin, Ruter et al. 1993). Another aspect, no considerable differences in soil density between layer 3 and 4 was observed (**Table 3.5**). Regarding soil pH, although soil pH on treatment (applied nutrient) in three first layers was significantly higher than on control (without nutrient), no significant difference in Geohumus WHC two in first layers between control and treatment was found. This could be attributed to the effect of salt on WHC of Geohumus in soil environments, a more important factor than soil density and pH soil as well. It could be concluded that salt accumulation from top layer resulted in decrease of WHC of Geohumus in lower layers.

4.1.5 Geohumus restorability (from soil and different salt solutions)

a) Used Geohumus (from soil and compost)

The results showed that Geohumus restorability in sandy with base nutrient and compost soil was too low while that in sandy soil with divided application of fertilizer was still quite high (**Fig. 3.6**)**.** Meaning that both soil type and fertilization method affected on WHC of Geohumus due to impacts of salt or elements mentioned in many earlier reports (AI-Darby, Mustafa et al. 1990; Martin, Ruter et al. 1993; Akhter, Mahmood et al. 2004; Dorraji, Golchin et al. 2010) e.g. salts in sandy soil from nutrient application and its property while composts from its property**.** Another aspect, although Geohumus was collected from the same media (sandy soil) there was significant difference in Geohumus recovery between Geohumus from base fertilizer application and from divided application of fertilizer (**Fig. 3.6**). Nutrient solution was mixed once at pot filling leading to Geohumus being in contact with high salt concentrations (**Section 2.2.2**) whereas Geohumus was in contact with salts to a much lesser extent due to the application of nutrient solution in an interval of three days (**section 2.3.2**). In short, soil type and method of nutrient application had strong effects on WHC of Geohumus. Divided application of fertilizer could be a feasible way to increase Geohumus WHC.

b) Used Geohumus (from different salt solutions)

We assume that when it rains under field condition, Geohumus can restore its WHC because soil solution is diluted, leading to a decrease in EC or salt concentration. An extra trial was conducted in the lab aiming to investigate this assumption. Geohumus was immersed in specific salt solutions divided into two subgroups, namely: group I (KNO_3 and NH_4NO_3) and group II ($FeSO_4$ and $Al_2O_{12}S_3$). Group I and group II represent chemical compounds that can hinder water absorption of Geohumus least and most, respectively.

Hydration of polymers is reduced by competition between water molecules and ions in soluble media, leading to impaired electrical repulsion of polymer chains (James and Richards 1986; Martin, Ruter et al. 1993). Further, bonds between charge groups in polymer chains and multivalent ions are more stable than those of monovalent ions (Chen, Zommorodi et al. 2003), which is in agreement with our results. In fact, group II could impede expansion much more strongly than group I, but the recovery of its WHC was much lower (**Table 3.4**). Bonds among Geohumus and multivalent ions in group II may be too strong as that these ions could be released to soluble media and thereby enabling the attachment of water molecules to Geohumus. This process is completely reversed for group I. This is the reason for the significant difference observed between group I and group II regarding the restorability of Geohumus in water. Finally, the higher the concentration was, the lower was the restorability of Geohumus (**Table 3.4**). The release of ions from polymer chains to solution depends on two conditions, i.e. the strength of bonds between ions and the charge group of the polymer and the ionic concentration of the soluble environment. If the ionic concentration is low and bonds are weak, Geohumus can be restored better, as the ions bonded to the polymer chains of Geohumus could be released more easily.

Based on results gained from this section, to improve WHC of hydrogels or Geohumus under field condition, understanding climate condition, soil water sources is very important. Reducing ionic concentration from soil, water and division of fertilizer application could be improved WHC of polymers.

4.1.6 Soil density, suction and moisture as influenced by treatments

a) Soil bulk density

Applying hydrophilic compounds to soil could change soil physical properties due to the expansion of the compounds affecting soil moisture, porosity, and hydraulic conductivity (Bai, Zhang et al. 2010). Previous results illustrated that the change of soil bulk density after application of polymers depended on soil moisture conditions (Bai, Zhang et al. 2010) as well as polymers impact on soil density depends on the polymers properties. The results presented here show that Geohumus application at a rate of 2.86 g kg soil^{-1} did not affect soil bulk density compared to a control without Geohumus application (**Table 3.5**). This was in agreement with previous a previous report where 0.2% of Polyacrylamide were applied to a silt loam soil (Steinberger 1990). However, earlier researches demonstrated that the application of Super- absorbent polymers at rates of 0.05-0.3% (Bai, Zhang et al. 2010) or polyacrylamide at rates of 650kg ha^{-1} (Terry and Nelson 1986) improved soil bulk density considerably. In this research, the low water absorption of Geohumus is one of the reasons why no significant difference in soil bulk density was observed between the control and Geohumus treatment.

b) Soil Matrix potential

Geohumus could slightly increase soil moisture at field capacity. However, at pF equal 4.2 considered as permanent wilting point Geohumus seemed to hold water in its own chains firmly when soil became drier leading to the effect that plants started wilting even though the soil moisture content was still higher compared to the control (**Table 3.6**). In this respect Geohumus seems to be comparable with Stockosorb as the results presented here on Geohumus are consistent with a report of Hüttermann and Zommorodi (1999) that showed that water absorbed by 0.4% Stockosorb K400 is not fully plant available.

c) Soil moisture

Literature showed that hydrophilic polymers had significant increase of soil water content because of their water absorption capacity (Chen, Zommorodi et al. 2003; Green, Foster et al. 2004; Abedi-Koupai and Asadkazemi 2006; Abedi-Koupai, Sohrab et al. 2008; Andry, Yamamoto et al. 2009; Dorraji, Golchin et al. 2010). Our results showed that whether soil moisture improvement of Geohumus was dependant on not only method of fertilizer application but also soil type e.g. Geohumus application to sandy soil with base nutrient application (drought spell experiment) could not increase soil moisture compared to control (**Fig. 3.7**) while Geohumus application to sandy soil with the divided application of nutrient could improve significant soil moisture (split root system experiment) (**Fig. 3.6**). Addition, compost soil had stronger in impeding Geohumus water absorption than sandy soil with the divided application of nutrient (**Fig. 3.6**). That could be attributed to salt concentration (**Table 2.1 and fig. 3.4**). There was negative relationship between salt concentration and Geohumus water absorption mentioned in **section 4.1.3 a**.

4.2 Morphological and physiological responses of two maize cultivars under prolonged water deficit as influenced by Geohumus application

4.2.1 Genotypic responses to water deficit

This section focuses on the analysis of growth responses, root-shoot partitioning, non-hydraulic signals, water status, leaf gas exchanges and water use efficiency of two maize cultivars to prolonged drought grown in sandy soil without Geohumus application. Mikado and Companero responded to progressive soil drying with a decrease in leaf area, root water potential, leaf water potential, leaf osmotic potential, root osmotic potential, and gas exchange (Gs, A, E) and with an increase of $[ABA]_{leaf}$, $[ABA]_{xylem}$, water use efficiency (WUE), and penetration depth of roots.

A reduction in leaf area under drought conditions was reported earlier (Yang, Fan et al. 1993; Khan, Hussain et al. 2001) showing that leaf area of plants cultivated on polymer-untreated soil correlated negatively with drought stress levels. Leaf area reductions could be due to a decrease of leaf elongation which in turn could be due to reduced water conductance through the root system (Tang and Boyer 2008) or a decrease in any tissue water potential (Hsiao and Acevedo 1974). That is consistent with the results presented here showing reduced root water potential values under drought (**Table 7.11 and 7.12**). Reductions in soil moisture lead to lower leaf water potentials (Harris and Health 1981; Sanchez, Hall et al. 1983; Bahrun, Jensen et al. 2002; Abdulai 2005; C.Dodd, Egea et al. 2010) and increases of $[ABA]_{leaf}$, and $[ABA]_{xylem}$ (Bahrun, Jensen et al. 2002; Asch, Bahrun et al. 2009). The increase of [ABA] and reduction in leaf water potential result in a decrease of gas exchange (Turner, Begg et al. 1978; Harris and Health 1981; Dodd, Tan et al. 2003). This was found true in the data presented here for both cultivars of maize, Mikado and Companero (**Table 7.11 and 7.12**). In addition, the two cultivars also showed the same responses, positive correlation between not only leaf water potential and leaf osmotic potential but also root water potential and root osmotic potential (**Fig. 3.15**). In that, both Mikado and Companero showed the ability to tolerate drought and maintain growth under drought condition (Gebre and Tschaplinski 2000). Positive close relationship between leaf water potential and leaf osmotic potential was illustrated by previous reports (Turner, Begg et al. 1978; Sharp and Davies 1985; Khan, Hussain et al. 2001). Both cultivars responded to drought by partially closing stomata resulting in an increase in WUE (**Table 7.5 and 7.6**). This occurs when the net assimilation rate is still higher than transpiration, even though both are limited by moderate water shortage whereas net assimilation rate ceases completely under extreme drought (Xu, Zhou et al. 2010).

There were different responses to drought between the two cultivars. Firstly, leaf weight in Companero was reduced after 9 days of drought along with decreasing leaf area while leaf weight in Mikado subjected to 8 days of drought was stable although its leaf area was reduced (**Table 7.5, 7.6**); Changes in specific leaf area (SLA) as observed in the present study in Mikado, were reported earlier (Marron, Dreyer et al. 2003). Further, Mikado responded to drought with an increase in root-shoot ratio (**Fig. 3.12a**) due to increasing of root weight while maintaining shoot weight (**Fig. 3.8c, d**). The higher SLA in Mikado allowed maintaining leaf water balance and thus a better maintenance of leaf photosynthesis in response to drying soil (Kishitani and Tsunoda 1982). In contrast, Companero responded to drought by reducing both shoot and root weight thus maintaining a similar root-shoot ratio as under well watered conditions **(Table 7.6).** Previous reports showed that under drought conditions root-shoot ratios increase due to maintaining or increasing of root growth but decreasing shoot growth (Westgate and Boyer 1985; Zhang and Davies 1989). Compared to Companero, Mikado strongly responded to drought by increasing rooting depth (**Table 3.7**) and redistributing of assimilates between leaves, stem, and roots. For example, the partitioning coefficient for stem biomass continued to increase, partitioning to the leaves decreased, and partitioning to the roots dramatically increased in Mikado whereas the partitioning coefficients under drought remained similar in Companero (**Fig. 3.15).** Finally, in Companero gas exchange and plant water status responded earlier (at approximately 50 % soil loss) whereas in Mikado these changes occurred later (at approximately 64 % soil loss) (**Table 7.13**). This could be the reason why Mikado maintained shoot weight during the drought spell whereas in Companero shoot weight was reduced (**Fig. 3.8 c**).

It has been reported earlier that tolerant genotypes of maize respond to drought by improving the root system in order to increase water and nutrient uptake (Vamerali, Saccomani et al. 2003) and sensitive cultivars respond to drought early through a decrease in gas exchange (Guóth, Benyó et al. 2010).

4.2.2 Genotypic responses to Geohumus under fully watered conditions

Under fully watered conditions, Geohumus had no effect on pH_{xylem}, $[ABA]_{leaf}$, $[ABA]_{xylem}$, root water potential, leaf water potential, leaf osmotic potential and xylem osmotic potential in either Mikado or Companero grown on sandy soil (**Fig. 3.14, 3.15**). This indicates that Geohumus application did not improve mean soil moisture content (**Fig. 3.7**). However, Geohumus application resulted in significant improvements in leaf area and leaf and shoot weight in both Mikado and Companero (**Fig. 3.8 a, b, c**) as well as increased photosynthesis rate (**Fig. 3.16 c**) resulting in greater biomass accumulation. It is well possible, that Geohumus application contributed to the nutrient availability of the cultivars as has been shown before for hydrophilic polymers that enhanced N, P, and Fe uptake (Magalhaes, Wilcox et al. 1987) as well as for Agrosoak that had positive effects on the content of Na, N, K in the leaves of maize (Silberbush, Adar et al. 1993b; Mikkelsen 1994). It was also shown earlier, that the application of Geohumus to a sandy soil led to increased nitrogen uptake under field conditions (Trimborn, Heck et al. 2008).

Geohumus application improved root growth of both cultivars under fully watered conditions (**Fig. 3.8 d**). Whereas total root weight was increased in both cultivars as compared to growing in soils with no Geohumus applied, roots of Mikado extended into deeper soil layers whereas roots of Companero were mainly found in the upper soil layers **(Fig. 3.9 and Table 3.7).** This was accompanied by increased root diameters and decreased root length density in Companero as compared to Mikado (**Fig. 3.9 and 3.11**). It has been reported before, that hydropolymer application results in improved root growth (Hüttermann, Zommorodi et al. 1999; Chen, Zommorodi et al. 2003; Koudela, Hnilička et al. 2011), however detailed responses as reported here are not found in literature to date.

4.2.3 Geohumus effects on genotypic responses to prolonged drought

Geohumus application in combination with drought affected the two cultivars differently. Whereas in Mikado an increase in leaf weight, leaf area, and stem weight was observed that resulted in an overall increase in shoot weight no effect on these parameters was observed in Companero (**Fig. 3.8 a b, c and Table 7.5, 7.6**). Earlier reports showed a positive correlation between nitrogen supply and shoot weight and leaf gas exchange depending on the genotype (Kishitani and Tsunoda 1982; Shangguan, Shao et al. 2004). Equally, soil nitrate deficiency has been shown to induce stomatal closure and reductions in leaf growth (Wilkinson, Bacon et al. 2007). In the present study Geohumus application had no positive effect on gas exchange in either cultivar under drought stress (**Fig. 3.16**). Thus the greater shoot weight of Mikado may have been the result from increased nitrogen uptake due to the Geohumus application (Trimborn, Heck et al. 2008). Photosynthesis rate under moderate stress was associated with leaf nitrogen content (Kishitani and Tsunoda 1982). It is possible, that Mikado profited from improved nitrogen availability due to the application of Geohumus already before the onset of the drought stress, resulting in increased water use efficiency when the plants were subjected to drying soil (**Fig. 3.12 b**).

The larger leaf area of both cultivars grown in sandy soil with Geohumus applied lead to a more rapidly decreasing soil moisture content and thus to higher stress levels which in turn resulted in an increase of $[ABA]_{leaf}$ and $[ABA]_{xylem}$ (**Fig. 3.14**) (Zhang and Davies 1989; Christmann, Weiler et al. 2007; Asch, Bahrun et al. 2009). This effect is consistent with results presented by (Moslemi, Habibi et al. 2011) since the plants would falsely respond to soil water content stored in the polymer and not to the average moisture content in the soil column resulting under prolonged stress conditions in reduced root growth or root elongation as compared to plants growing without polymer application (**Fig. 3.8 d, 3.10, 3.11**) (Robertson, Yeung et al. 1990; Deak and Malamy 2005). Although, the results presented here contradict previous reports (Chen, Zommorodi et al. 2003; Shi, Li et al. 2010; Koudela, Hnilička et al. 2011) showing positive effect on roots of some plants grown in treated polymer-media due to positive contribution of soil moisture it is important to note that Geohumus did not improvement soil moisture content in the present study (**Fig. 3.7**).

Mikado and Companero also responded to Geohumus application by re-partitioning assimilates from roots to shoot when subjected to drought, resulting in lower root-shoot ratios (**Fig. 3.12 a**). Geohumus, as the considered hydrophilic polymer, could improve nutrient uptake, i.e. nitrogen and phosphate (Magalhaes, Wilcox et al. 1987; Mikkelsen, Jr. et al. 1993; Silberbush, Adar et al. 1993b). Increasing nitrogen concentration increased dry matter accumulation, resulting in reduction of root shoot rate (Cechin 1997). This is consistent with our result since both cultivars, Mikado and Companero, maintained leaf growth while root growth was reduced (**Fig. 3.8, 3.10**).

4.2.4 Genotypic responses to re-watering after a drought period as influenced by Geohumus

Up to now, little is known about effect of polymers on plant physiology, morphology, and water status under re-watering after subjected to prolonged drought. The results presented here show that pH_{xylem}, $[ABA]_{xylem}$, leaf and root water potential, leaf and xylem osmotic potential, transpiration, stomatal conductance, root distribution and partitioning coefficients of the two cultivars supplied with Geohumus and subjected to drought recovered to control values after re-watering (**Fig. 3.13 c, d, 3.14 a, c, 3.15 a, b, c, d, 3.16 a, b and Table 3.7**). The increase in root water potential **(Fig. 3.15)** resulted in greater Companero leaf area due to the recovery of leaf elongation (Hsiao and Acevedo 1974; Tang and Boyer 2008) and of cell area (Lechner, Pereyra-Irujo et al. 2008) when fully watered conditions were re-established. Consequently, SLA was reduced in Companero as compared to control while SLA in Mikado did not respond to re-watering (**Fig. 3.8 a, b)**.

Re-watering also re-established plant nutrient absorption from the soil (and Geohumus) leading to increased photosynthesis rates in both cultivars. However, the recovery of Companero $[ABA]_{leaf}$ to control levels was faster than in Mikado resulting in comparatively higher photosynthesis rates (**Fig. 3.14 b and 3.16**). The negative effects of drought on leaf area, leaf weight, shoot weight, and root weight were not compensated before 15 after re-watering as compared to the full irrigation treatment (**Fig. 3.8**).

4.3 Effects of Geohumus and soil type on drought induced root-shoot communication of genotypes

In the previous section (**section 4.2**), the effects of Geohumus on morphological and physiological responses of maize under prolonged dehydration were discussed. In this part, the discussion focuses on interactions of soil type and Geohumus application on drought induced root-shoot communication as indicated by changes in non-hydraulic, stomatal conductance and plant water status parameters.

4.3.1 Effects of soil type on drought induced root-shoot communication of genotypes

a) Root water potential in split root systems ensuring root-shoot communication

Water potentials of different plant organs are indicators of water stress. Decreasing root water potentials indicates soil water limitations. Maize grown in both sandy soil and compost under partial root zone drying had lower root water potentials compared to full irrigation but higher than deficit irrigation (**Table 3.12**); meaning that the process of non-hydraulic and hydraulic root-shoot communication could take place (Wagdy Y. Sobeih, Dodd et al. 2004). The following paragraphs will discuss this process.

b) Effect of soil type on hydraulic root-shoot communication

Leaf water potential of maize on sandy soil under partial root zone drying was in between that under full irrigation and that under deficit irrigation. It was shown by some previous reports that in split root systems plants are able to switch their pattern of water absorption from the dried to the wet compartment (Green and Clothier 1995; Yao, Moreshet et al. 2001; Liu, Song et al. 2008). Compensatory water uptake from the wet part of the root system maintained leaf water potential of Mikado (Lawlor 1973; Blackman and Davies 1985). However, leaf water potential of Mikado grown on compost under partial root zone drying was not maintained similar to under full irrigation (**Table 3.11**). It could be interpreted that the compensatory effects observed in our study did not last long due to the limited water resources at the wet side (**Table 3.12**). The larger leaf area of Mikado grown in compost than in sandy soil leading to more water consumption could be to the major reason (**Table 3.16**) although the water content of the sandy soil was lower than that of compost (**Table 2.6**).

c) *Genotypic response to hydraulic root-shoot communication*

Similar to Mikado grown in compost, the leaf water potentials of Companero grown on sandy soil was affected by partial root zone drying; meaning that hydraulic root-shoot communication was limited due to plant subjected to severe drought conditions (Dodd, Egea et al. 2008). When grown under the same conditions (sandy soil), however, Mikado leaf water potential under partial root zone was unaffected (**Table 3.11**). Again, Companero was more affected by drought stress than Mikado due to a larger leaf area (**Table 3.11, 3.12);** that resulted in Companero hydraulic root-shoot communication under partial root zone drying affected due to more water consumption. It could be concluded that difference of genotypic response to media; the greater leaf area resulted in limitation of hydraulic root-shoot communication under partial root zone drying.

d) Effect of soil type on non-hydraulic signal root-shoot communication

An apparent evidence of partial root zone drying shows that the wet side of the root system compensates water uptake to the shoot to maintain shoot water status (Blackman and Davies 1985). The drying side of roots produces chemical signals (ABA) to control stomatal conductance (Loveys 1984). The data presented here indicates a stronger correlation of $[ABA]_{xylem}$ on both sandy soil and compost with stomatal conductance than $[ABA]_{leaf}$ (**Table 3.9, 3.10, 3.15**). This result is in agreement with some evidence from Literature (Loveys 1984; Zhang and Davies 1989; Stoll, Loveys et al. 2000). Other reports demonstrated how plant [ABA] plays a role in the regulation of stomatal conductance, for example in tobacco $[ABA]_{xylem}$ affected root osmotic potential to regulate stomatal conductance (Mizrahi, Blumenfeld et al. 1970) while in bean $[ABA]_{leaf}$ also contributed to leaf osmotic adjustment (Güler, Sağlam et al. 2012). However, these results came from two different experiments with two different species. Thus, it is hard to define whether $[ABA]_{leaf}$ or $[ABA]_{xylem}$ affected osmotic potential. In the present study, the relationship between ABA concentration, osmotic potential, and stomatal conductance under different water availability , indicates that $[ABA]_{xylem}$ on compost soil affected root osmotic potential and stomatal aperture **(Table 3.10, 3.14, 3.15)**, whereas

$[ABA]_{xylem}$ on sandy soil regulated stomatal aperture and was related to leaf osmotic potential **(Table 3.10, 3.13, 3.15).** Transporting $[ABA]_{xylem}$ from roots to shoot to regulate stomatal conductance has been established as a root signal in drying soil (Zhang and Davies 1989; Stoll, Loveys et al. 2000; Wagdy Y. Sobeih, Dodd et al. 2004).

e) Genotypic response to non-hydraulic signal root-shoot communication

Genotype could play a role in controlling stomatal conductance under drought stress via non-hydraulic signals. This was demonstrated in an earlier report e.g. both wild-type and transgenic plants of tomato had a close relationship between pH_{xylem} and stomatal conductance after 2.5 days subjecting to partial root zone drying. But only for the wild-type tomato a correlative relationship between stomatal conductance and $[ABA]_{xylem}$ was found (Wagdy Y. Sobeih, Dodd et al. 2004). Another authors reported that an increase of $[ABA]_{leaf}$ affected root osmotic potential resulting in reduction of transpiration (Mizrahi, Blumenfeld et al. 1970). However, within 40 hours subjecting to different levels of water supply, our result showed that Mikado and Companero grown in sandy soil had the same response i.e. $[ABA]_{xylem}$ regulated stomatal closure (**Table 3.10, 3.15**) by reducing leaf osmotic potential (**Table 3.12**).

In summary, without Geohumus application, plant water and non-hydraulic root-shoot communication depended on soil types and genotype.

(1) hydraulic root-shoot communication:

- Greater leaf area of Mikado grown in compost resulted in limitation of hydraulic root-shoot communication compared to when grown in sandy soil.

- On sandy soil, larger leaf area of Companero also caused the limitation of hydraulic root-shoot communication compared to Mikado.

(2) non-hydraulic root-shoot communication: $[ABA]_{xylem}$ of Mikado (on sandy soil and compost) and Companero (on sandy soil) moved to shoot to regulate stomatal conductance. However, Mikado $[ABA]_{xylem}$ under more severe drought stress affected stomatal conductance followed by root osmotic potential whereas Mikado $[ABA]_{xylem}$ regulated stomatal conductance followed by leaf osmotic potential.

4.3.2 Effect of soil type with Geohumus applied on drought induced genotypic root-shoot communication

a) Geohumus application increasing soil [ABA]?

The presence of Geohumus in both media (sandy soil and compost) tended the increase of $[ABA]_{xylem}$ of Mikado and Companero. As discussed in drought spell experiment, Geohumus application caused higher $[ABA]_{xylem}$ due to greater leaf area. That is also true for the results obtained in the split root system experiment (**Table 3.10, 3.16**). However, higher $[ABA]_{xylem}$ with Geohumus was observed under both deficit irrigation and full irrigation (**Table 3.10**). It allows inducing that $[ABA]_{xylem}$ of both cultivars came from out of the endogenous source. An evidence should be considered that un-watered plant could absorb ABA from soil released by microorganisms (Davies and Zhang 1991) and polymer was considered as an organic source for soil microorganisms (Kay-shoemake, Watwood et al. 1998). Thus $[ABA]_{xylem}$ on Geohumus application treatment could involve additional [ABA] from soil.

b) Effect of soil type with Geohumus on hydraulic root-shoot communication

Under full irrigation, circulation of water between root and shoot could take place easily (Loveys 1984). However, this process is limited when plants are subjected to severe drought (Dodd, Egea et al. 2008). The assumption is that Geohumus application to the soil would improve hydraulic root-shoot communication. Thus leaf water potential under partial rootzone drying with Geohumus applied would be higher and closer to full irrigation as without Geohumus application. However, the results shown here were opposite. It could be interpreted that although Geohumus application to sandy soil increased both soil moisture and leaf area (**Table 2.6, 3.16**) the extra amount of water from Geohumus application could not compensate for the higher water consumption due to the greater leaf area. Consequently, Geohumus application to sandy soil resulted in a reduced leaf water potential under partial rootzone drying (**Table 3.11**). In contrast, when grown on compost soil with Geohumus applied the leaf water potential under partial rootzone drying was unaffected (**Table 3.11**). It is in agreement with previous reports (Lawlor 1973; Blackman and Davies 1985; Saab and Sharp 1989; Dry and Loveys 1999) that showed that plant leaf water potential under partial rootzone drying is similar to that under full irrigation. Actually, on sandy soils with Geohumus applied soil moisture was not increased (**Table 2.6**) but leaf area was reduced (**Table 3.16**). Smaller leaf area reduces water consumption leading to a better water balance between the dry and the wet side of the root system. This result is in agreement with earlier research (Flannery and Busscher 1982) that showed that Perabsorb mixed with peat decreased shoot biomass of Azalea and Impatiens due to increase of field capacity leading to less oxygen supply to the roots. In summary, leaf area showed a different response to the combination of soil type and Geohumus application. Geohumus application to sandy soil improved leaf area but Geohumus application to compost soil reduced leaf area. These differences in Leaf area also resulted in differences in root-shoot communication.

c) *Effect of Geohumus application on genotypic responses to hydraulic root-shoot communication*

Geohumus application to sandy soil improved the water content and the leaf area in both cultivars (**Table 2.5, and 3.16**). In addition, in both cultivars leaf water potential on sandy soil with Geohumus applied was reduced but was not significantly different under partial root zone drying and deficit irrigation as compared to full irrigation (**Table 3.11**). These results disagree with some earlier reports (Lawlor 1973; Blackman and Davies 1985; Saab and Sharp 1989; Dry and Loveys 1999). It is possible that applying Geohumus to sandy soil resulted in larger leaf areas. That accounts for negative effects on hydraulic root-shoot communication under partial root zone drying.

d) Effect of Geohumus on non-hydraulic signaling from root to shoot

Geohumus application to sandy soil and compost resulted in higher $[ABA]_{xylem}$ in both cultivars. However, there was no close correlation between $[ABA]_{xylem}$ and stomatal conductance (**Table 3.10, 3.15)**. It appears that Geohumus application to sandy soil disabled chemical signaling from roots to shoot. It is possible, that the circling of [ABA] between roots and shoot through xylem and phloem under well watered condition (Loveys 1984), is decreased when the plant grows under severe drought (Tyree and Dixon 1986; Sperry and Tyree 1990; Dodd, Egea et al. 2008) due to interruptions or embolies in the xylem conduit (Sperry and Sullivan 1992). In general, it would have been expected, that an increased xylem ABA concentration leads to faster and more efficient stomatal closure under drought conditions.

CONCLUSIONS

Drought is among the most detrimental threats agriculture has to face, particularly in many tropical and subtropical countries. Improving soil, crop, and irrigation management offers some possibilities to mitigate drought effects and to achieve stable yields. Among others, synthetic polymers, such as Geohumus, are used for soil amelioration measures to improve crop water availability; however, our understanding about how Geohumus interacts with different environments and affects plant's physiological responses to stress is still limited. Thus, the aim the research presented here was to i) investigate the effects of selected abiotic factors on the water holding capacity (WHC) of Geohumus, and ii) to analyze the effects of Geohumus application to the soil on crop genotype responses to drought.

Under laboratory conditions Geohumus has a WHC of about 40 times its weight. However, when tested in solutions differing in salt concentration and salt type WHC of Geohumus was drastically reduced. This effect was stronger when multivalent rather than monovalent ions were dominating. The reduction in WHC occurred not only in solution, but also in sandy soil and compost soil with standard fertilizer application. In addition, WHC of Geohumus was strongly influenced by temperature. Under low temperatures WHC of Geohumus was strongly reduced, whereas high temperature conditions increased WHC of Geohumus. With the increase of soil WHC being the main justification for applying Geohumus to field crops, these results clearly reveal, that Geohumus application to a normal soil, particularly in temperate climates, will not achieve the desired effect. However, the reduction in WHC of Geohumus could be partially off-set when fertilizer application was split into several applications in order to reduce the actual concentration of salts in the soil solution.

Trying to restore the water absorption rate of Geohumus after incorporation into the soil or after imbibing it in different salt solutions clearly revealed nutrient absorbing properties of Geohumus. The WHC of Geohumus was only partly restored, however, Geohumus may play a role as soil colloid mitigating nutrient losses (particularly in sandy soils) by absorbing and releasing nutrients for plants to take up and thus improving crop nutrient use efficiency, particularly in nutrient limited systems or soils.

The nutritional effect of Geohumus was tested via application of Geohumus to a sandy soil under fully watered conditions. Both maize cultivars responded with an increase in total biomass, although Geohumus application did not increase soil water content, indicating, that Geohumus improved the nutrient availability for plants thereby boosting aboveground productivity. The larger shoot biomass, and in particular the thus larger leaf area, induced by application of Geohumus before the on-set of drought, resulted in increased water uptake of plants growing in soils with Geohumus applied when they were subjected to drought conditions. Both maize genotypes were thus exposed to a more severe stress situation with Geohumus applied which is reflected in a reduced water potential and by higher concentrations of $[ABA]_{leaf}$ and $[ABA]_{xylem}$, as well as reduced root length and final biomass as compared to plants without Geohumus applied. Overall, and this was also shown in split-root experiments here, drought responses were strongly influenced by genotype and then modified by the presence or absence of Geohumus in the root zone. Geohumus application modified some of the internal regulatory mechanisms for water balance and stomatal conductance of the tested genotypes but did not improve genotypic performance under drought under any conditions. These results provide the first scientific evidence, that application of Geohumus to field crops neither improves plant water availability in drought situation, nor mitigates drought effects on plant growth. The data presented here prove a stress enhancing effect of Geohumus application on maize under drought. It has been shown here, that the reason for the poor performance of Geohumus in plant cultivation lies within the physical properties of Geohumus itself, rendering its use in agriculture doubtful. For the mitigation of drought stress by increasing water available a polymer or organic hydro-absorber should be used whose WHC is not reduced by salts in the soil solution, does not increase fertilizer effects under drought conditions and which is restorable to the original properties after rewatering. The interaction between genotypic drought responses and any potential soil ameliorant is highly important to evaluate to what extend the physiological responses of the genotype may interfere with the intended use of the hydro-absorber. Ideally such a product should be targeted to a specific crop or to a specific cropping system. Thus, further research is needed into economically viable soil ameliorants actually promoting plant growth under water limited conditions and thus allowing more efficient use of the precious resource water across a wider range of cultivation systems.

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