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**Climate change-induced abiotic stress affects
agriculture**

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General introduction

Research on the impacts of climate change on agriculture, in general, and rainfed agriculture, in particular, has been largely deficient, except till very recently. However, recently generated knowledge on the subject provides a strong basis for compiling whatever work has been done in the field and disseminating it on a large scale. Aspects related to abiotic stress tolerance and how this would be affected by climate change is a major step forward in improving our understanding of the subject. It is significant that hundreds of millions of small farmers across the world, who are largely dependent on rainfed agriculture already face a number of stresses resulting from the very nature of their activities and the implications for livelihoods dependent on rainfed agriculture are of great significance. The impacts of climate change generally tend to exacerbate these abiotic stresses.

In both natural and agricultural conditions, plants are frequently exposed to environmental stresses. Some environmental factors, such as air temperature, may become stressful in just a few minutes; others, such as soil water content, may take days to weeks, and factors such as soil mineral deficiencies may take months to become stressful. It has been estimated that because of stress resulting from climatic and soil conditions (abiotic factors) that are suboptimal, the yield of field-grown crops in the United States is only 22% of the genetic potential yield (Boyer, 1982). In addition, stress plays a major role in determining how soil and climate limit distribution of plant species. Thus, understanding the physiological processes that underlie stress injury and the adaptation and acclimation mechanisms of plants to environmental stress is of immense importance to both agriculture and the environment.

Stress is usually defined as an external factor that exerts a disadvantageous influence on the plant. This short review will concern itself with environmental or abiotic factors such as heat, drought, salt, heavy metal and lack of nitrogen, that produce stress in plants, although biotic factors such as weeds, pathogens, and insect predation can also produce stress. In most cases, stress is measured in relation to plant survival, crop yield, growth (biomass accumulation), or the primary assimilation processes (CO₂ and mineral uptake), which are related to overall growth. In the next few pages, state of the art in Europe, main effects on crops and the strategies that plants use to mitigate stress conditions, will be briefly explained.

1. Drought stress

Europe's three major climatic zones are determined by its latitude, topography, distance to the sea, ocean currents and winds. First of all, southernmost Europe has a Mediterranean climate with mild temperatures, dry summers and wet winters. However, geographical factors within this region, such as distance to the sea and topography can, in little distance, harsher weather conditions. For instance, the Mediterranean watershed of the Iberian Peninsula has the lowest recorded precipitation in Europe, Cabo de Gata <150mm/year; whereas nearby zones can record greater amounts in just 24 hours (Olcina, 2001). Moreover, northwestern parts of the continent are characterized for a Temperate Oceanic climate influenced by the Gulf stream and North Atlantic Oscillation, with mild temperatures, high for its latitude, and constant precipitation throughout the year. Thirdly, northeastern parts of Europe have a Temperate Continental climate with cold winters and mild summers. In fact, its distance from the sea and influence of Siberian air masses can result in intra-annual marked temperature differences. Finally, it is important to highlight that these three climatic zones are, in all cases, disrupted by the display of Europe's main mountain ranges. For example, the Alps, Scandinavian, Pyrenees and Carpathian Mountains, have severe weather conditions typically of subarctic climates (Kottek et al., 2006).

As a consequence of Europe's diverse climatic configuration, agriculture its constraint by the previous but, recent agronomic innovations have certainly altered conventional agriculture. For instance, The Netherlands is recently placed among the countries with greatest vegetable production. Despite of the latter, Mediterranean agriculture is traditionally characterized for large plantations of olive, fruit and citrus trees, grapes and vegetables, mainly found in Italy, France and Spain. In addition, the central-north European belt mainly produces cereals, wheat, maize and barley; being France, Germany, Poland and the UK the countries with greater cereal production. Other eastern countries, such as Romania and Bulgaria, continue to lead the continent's sunflower production (EC, 2013).

Water stresses and its impacts have been in the scope of research for decades, but are interpreted in different ways according to the region, need and disciplinary approach. In fact, until the 1980's more than 150 definitions of drought can be identified. The work of Wilhite and Glantz (1985) compile all definitions of drought, while dividing them into four categories according to its implications and duration: meteorological, agricultural, hydrological and socio-economic. Meteorological drought is defined as the degree of dryness, in comparison to a long-term average, and duration of the dry period. Agricultural drought describes the difference between water demand and soil water availability (Kulik, 1962). Furthermore, hydrological drought is commonly related to the state of surface and underground water, where its supply is insufficient under specific water management strategies (Linsley et al., 1958). Finally, socio-

economic drought incorporates aspects of the aforementioned types of drought, where water supply is not adequate to meet the needs of certain human activities (Hoyt, 1942).

Discrepancies among researchers on defining drought are polished in the World Meteorological Organization (WMO, 2016) handbook, where a list of drought indices are giving according to their characteristics, input parameters, applications, strengths and weaknesses. In regards to agricultural drought, increasing efforts are made to identify the most appropriate indices (WMO, 2010). So far the following drought monitoring indices are extensively used as Early Warning Systems (EWS) in many countries.

The Standardized Precipitation Index (SPI) McKee et al., (1993 and 1995) is one of the most widespread index, characterizing meteorological drought on a range of timescales. The SPI is the number of standard deviations that observed cumulative precipitation deviates from the mean average. As a result, the SPI is a very versatile tool that can be calculated at any timescale while being suitable for hydrological and agricultural purposes.

$$SPI = \frac{(X_i - \bar{X})}{\sigma}$$

X_i : Recorded precipitation (year of study)
 \bar{X} : Mean Total precipitation (all years)
 σ : Standard deviation(all years)

Classification	Value
Mild drought	<0 SPI > -1
Moderate drought	> -1 SPI < -1.5
Severe drought	> -1.5 SPI < -2.0
Extreme drought	SPI < -2.0

Fig.1. SPI classification (McKee et al.,

The Crop Moisture Index (CMI) Palmer (1968) can be used to monitor droughts, where agricultural impacts are the primary concern. The CMI depicts short term information, up to 4 weeks, of abnormal dryness or wetness affecting agriculture. Its main limitation is

Classification	Value
Excessively wet	3.0 and above
Wet	+2.0 to +3.0
Abnormally moist	+1.0 to +1.9
Slightly dry/moist	-0.9 to +0.9
Abnormally dry	-1.0 to -1.9
Excessively dry	-2.0 to -2.9
Severely dry	-3.0 or less

Fig.2. CMI classification (Palmer, 1968)

that it cannot be applied during germinating and non-depth rooted crops. It is used by the USA Drought Portal to monitor agricultural droughts.

Main drought effects on crops

Function of water in crops

Water is the most important component of crops representing between 70 to 90 % of the total plant weight. Crop consisting of total dry matter of 5 t ha^{-1} contains up to $20 \text{ m}^3 \text{ ha}^{-1}$. In fact, this amount of water hold in the plant is just 5% of the total water requirement during the growing season. At first, water is required for photosynthesis, where solar energy transforms water and carbon dioxide into carbohydrates while releasing oxygen. Another function of water in crops is turgidity, where sufficient water pressure is needed to keep the plant rigid. Nonetheless, if there is not enough water available for the plant-cells the plant wilts. Moreover, water is an optimal solvent for nutrients and therefore for its transport along the plant. However, most of crops water requirement comes from transpiration processes. In this case, water evaporates and gets out of the plant from the leaves into the air. During photosynthesis water is loss to the air via the stomata, thus cooling and preventing the plant from overheating, while keeping the optimal temperature range for biochemical processes. As a result, there is greater water loss via the stomata during dry and windy summer days than in wet and non-windy cold days (Kramer, 1995).

Water flow in the plant

Water is lost from the plant through transpiration at the leaves via de stomata thus leading to a water gradient along the plant, from low suction in the soil to higher in the air. Different water gradients results in water being transported from the soil into the leaves. In fact, the amount of water loss by transpiration depends on the evaporative demand of the air as well as the water supply from the soil. For example, it the soil water supply is low and the demand is high the plant wilts. Moreover, water flow from the soil to the leaves encounters several resistances in the tissues of the roots, stems and leaves. These resistances consequently slow down the transport of water throughout the plant. For instance, the differences between the suction power of the root and soil divided by the resistances found at the root determines the water flow within the roots. Such situation occurs in the leaves and stems, where water flow is determined by the difference of the suction power between the leaf-air and root-leaf, respectively divided by the resistance found at the leaf and stem (Fiscus, 1975).

Water demand: stomata regulation and evapotranspiration

First of all, stomata opens under the influence of sun-light, where carbon dioxide is transformed into carbohydrates and then transported by phloem vessels into other parts of the plant. On the other hand, water flows from xylem vessels into the stomata cavity, and once the cavity saturates water is lost through transpiration processes onto the outside air. If the outside air has very low

relative humidity the evaporative demand will be higher. Similar situation occurs during windy days, when relative humidity of the air surrounding the plants is lower while increasing the evaporative demand. In addition, temperature is also a determining factor of relative humidity because at higher temperatures air can contain greater amounts of water than during night and cold days. Finally, longer hairs within the boundary layer of the leaves can hamper the movement of water molecules during windy day, hence transpiration is lower in comparison to plants without hairs (Kramer, 1995).

Water supply: water balance

The water demand is determined by evapotranspiration. For potential production optimal water must be supplied to the crop, being the soil the most important medium for water supply. This supply can derive both from soil moisture content as well as the depth of the root zone. The major input of water for a crop is precipitation, however additional water can be supplied through irrigation. In addition, the type of rainfall and soil determines how much water reaches, through infiltration, the groundwater. For instance, during showers in clayey soils, with fine particles and narrow size pores, less water reaches the groundwater while most of it is lost through surface runoff and evaporation. The opposite occurs during drizzling days in sandy type of soil, where percolation is higher and capillary rise is lower (Kramer, 1995).

Water use: drought tolerance and water-logging

Crops can respond in many different ways to water constraints. If a dry spell occurs early in the growing season it takes longer for canopy closure, while in the meantime evaporation losses increase. A lower dry matter production implies less dry matter available for storage organs. Depending on the development stage and type of crop, drought can have different impacts on the dry matter fraction found in roots, stems, leaves and organs. As a result, drought reduces net assimilation, therefore the development stages of the plant and dry matter distribution. Water constraints can be mitigated through different methods such as surface flooding, sprinkler and drip irrigation systems. In the latter, water losses like percolation, evaporation and transpiration are minimized (Chapin, 1991). In regards to water logging, crop can also suffer from water surplus often occurring in areas with heavy showers and soils with low infiltration rates. Nevertheless, it can also happen when the subsoil has a non-permeable layer, thus the rooting zone fills up with water and no oxygen is available for the roots. In fact, in order for roots to take up nutrients and water oxygen is needed, thus hampering the plant's growth. To prevent water-logging in crops, ditches and drainage systems are often used (Justin, 1987).

2. Heat stress

Due to increased temperature by climate change heat stress is a growing agricultural problem in many areas in the world. Heat stress can be defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development. It is a complex function of intensity (temperature in degrees), duration, and rate of increase in temperature (Wahid et al., 2007). As mainly water stress is connected with temperature stress, leaf temperatures usually increase above air temperature as a result of stomata closure and reduced transpiration (Hatfield, 1979). The leaf temperatures under these conditions can limit dry matter accumulation because of increased respiration, reduced photosynthesis and cellular damage (Basra, 1993). Consequently heat stress, in particular during vegetative and reproductive stages, causes severe yield reductions in different crops (Pessaraki, 1999).

Table 1. Annual crop species adapted to cool and warm seasons (Hall, 2001).

Cool-Season Annuals	Warm-Season Annuals
Barley, brassicas, canola, fava bean, flax, garbanzobean, Irish potato, lentil, lettuce, lupine, mustard, oat, pea, radish, rye, spinach, triticale, turnip, vetch, wheat	Common bean, cotton, cowpea, cucurbits, finger millet, grainamaranth, lima bean, maize, mungbean, pearl millet, pepper, pigeonpea, rice, sesames, sorghum, soybean, sunflower, sweet potato, tobacco, tomato

Plants have an optimal temperature range for growth as well as for reproduction. In Tab. 2 temperature ranges for adaptation of cool-season and warm-season annual crop plants are provided. These estimates are very approximate, since these two groups contain many different species (Tab. 1), and extreme temperature limits depend on the duration of exposure and extent of hardening (Hall, 2001).

Table 2. Temperature ranges of adapted cool-season and warm-season crop plants (Hall, 2001).

	Night Temperatures °C		Day Temperatures °C	
	Minimum	Maximum	Optimum	Maximum
	<i>Freezing</i>	<i>Heat stress</i>		<i>Heat stress</i>
Cool-season annuals	-30 to -1	> 16 to 24	18 to 28	> 28 to 40
	<i>Chilling</i>	<i>Heat stress</i>		<i>Heat stress</i>
Warm-season annuals	<6 to 18	>20 to 30	26 to 36	>30 to 50

In this context a threshold temperature is often defined, as a value of daily mean temperature at which a detectable reduction in growth begins. Through controlled laboratory and field experiments upper and lower development threshold temperatures were found out for many plant species. Lower development threshold temperatures or base temperatures vary within plant species. For cool season crops 0°C is often the best-predicted base temperature (Miller et al., 2001). Upper threshold temperatures also vary for different plant species and genotypes within species. Still, determining a consistent upper threshold temperature is difficult because the plant behaviour may differ depending on other environmental conditions (Miller et al., 2001; Wahid et al., 2007). Upper threshold temperatures for some major crop species are shown in Table 3. In Europe high temperature sensitivity is particularly important in arid and semi-arid regions as heat stress may become a major limiting factor for field crop production.

Table 3. Threshold high temperature for some crop plants (Wahid et al., 2007).

Crop plants	Threshold temperature (°C)	Growth stage	References
Wheat	26	Post-anthesis	Stone and Nicolas (1994)
Corn	38	Grain filling	Thompson (1986)
Cotton	45	Reproductive	Rehman et al. (2004)
Pearl millet	35	Seeding	Ashraf and Hafeez (2004)
Tomato	30	Emergence	Camejo et al. (2005)
Brassica	29	Flowering	Morrison and Stewart (2002)
Cool season	25	Flowering	Siddique et al. (1999)
pulses	34	Pollen production	Vara Prasad et al. (2000)
Groundnut	41	Flowering	Patel and Hall (1990)
Cowpea	34	Grain yield	Morita et al. (2004)
Rice			

Main heat stress effect on crops

Since plants are unable of maintaining a temperature optimal for their growth, a slight increase in temperature can affect physiological and biochemical process crucial for plant growth. Plants are able to tolerate temperatures of 5-10°C above the optimal temperature without being stressed. At temperatures of 12-15°C beyond the optimal temperature, plants begin to suffer from heat stress. An unexpected increase in temperatures of 15°C or more above the optimal range can affect seriously the plant growth and development, depending

upon duration of the heat stress. Lethality results from a combination of cellular changes that the heat induces and the incapability to restore normal cellular function afterwards. Heat stress events cause irreversible damage during vegetative and reproductive stages in many crop plants. Some reasons are low photosynthetic activity, poor floral development, pollen sterility, which affects seed and fruit set as well as quality. High temperature affects numerous physiological activities associated with seedling growth and vigour, root growth, nutrient uptake, water relations of cells, solute transport, photosynthesis, respiration, general metabolisms, fertilization as well as maturation of fruits. The rate of photosynthesis in most species decreases at about 35°C which is attributed to protein denaturation, loss of membrane integrity, photo-inhibition and ion imbalance. High temperature has also an effect on chloroplast biogenesis and senescence, causes disintegration of chloroplast brings disruption of the structure of membrane protein, influences protein-lipid interactions, affects electron transport activity and substantially decreases the activity of Rubisco enzyme (Di Toppi and Pawlik-Skowrońska, 2003).

However, plants have the possibility to resist high temperature stress by avoidance or by tolerance mechanisms. So, heat stress does not mean immediately lethality. Examples of heat avoidance mechanisms are insulation, decreased respiration, decrease absorption of radiant energy through reflectance or decreased chromophore content, transpiration cooling (Levitt, 1980). Potential mechanisms of heat tolerance are the synthesis of protectants (Levitt, 1980), increased thermo stability of enzymes (Weber et al., 1977), and increased saturation of fatty acid (Basra, 1993). During period of heat acclimation, a set of novel proteins is synthesized; they so called heat-shock proteins (HSPs) (Vierling, 1991). These proteins are thought to enable cells to survive the harmful effects of heat by two general types of mechanisms: as molecular chaperones and by targeting proteins for degradation (Hall, 2001).

Strategies to mitigate heat stress

The adverse effects of heat stress can be moderated by developing crop plants with improved thermo tolerance using various genetic approaches. Nevertheless, not all plant species or genotypes within species have similar capabilities in coping with this abiotic stress. There exist remarkable differences within and between species, providing opportunities to improve crop heat-stress tolerance through genetic means. Some efforts to develop heat-tolerant genotypes via conventional plant breeding protocols have been successful (e.g. Ehlers and Hall, 1998; Camejo et al., 2005). Recently, innovative techniques of molecular breeding and

genetic engineering are additional tools, which can be used to develop crops with improved heat tolerance and to combat this universal environmental adversary. Though, to assure achievement of success in this strategy, concerted efforts of plant physiologists, molecular biologists and crop breeders are domineering (Wahid et al., 2007).

EU strategies to mitigate drought and heat stresses

Mediterranean countries are highly exposed to drought due to intra- and inter annual rainfall variability. Under changing climatic conditions increasing water shortages are expected to augment in frequency and intensity along southern and southeastern regions. However, longer drought-spells and raising temperatures, particularly in summer, are also projected to be more recurrent in northern parts of Europe (Jol et al., 2008). In fact, between the periods 1976-1990 and 1991-2006 the population and area affected in Europe has folded, while yearly costs of droughts have quadrupled (EEA, 2010). For instance, the 2003 heat-wave and drought affected most European countries, especially those within western, southern and central parts (Luterbacher et al., 2004). Excessive mortality during this heat-wave was unusual and alarming, with an estimated number of casualties of 52,000 up to 70,000, depending on the sources (Larsen, 2006; Robin et al., 2007).

Furthermore, the EU policy framework on droughts and heat-waves seeks to increase resilience amongst Europe's most exposed and susceptible human and environmental activities through preparedness, risk reduction and management plans. As a result, the EU has a wide range of interacting policy instruments, directive and communications, that aim to mitigate as well adapt critical sectors to forthcoming natural hazards. For instance, the Common Agriculture Policy (CAP), Floods Directive, EU Climate Adaptation Policy, EU Water Framework Directive, EC Communication 'Blueprint to Safeguard Europe's Water Resources' or the Heat Human Warning Systems (HHWS) as a consequence of the 2003 heat-wave (Lowe et al., 2011; Stein et al., 2016).

All of the above have a water-oriented perspective and being the CAP the main tool for agriculture financial support, while embracing a set of risk management strategies to address the impacts of climatic hazards (EC, 2012). However, CAP shows a vague approach on precautionary measures with no consideration to drought prevention. In the same line is the operational EWS for water-related hazards and the European Flood Alert System (EFAS), predominantly focused on risk reduction and response plans for water excesses (Alfieri et al., 2012). Furthermore, the poor scientific and political attention giving to the early warning of droughts is somehow mended through the EWS for meteorological droughts (Lavaysee et al.,

2015). In their study the SPI is incorporated to quantify the onset or likely evolution of an ongoing drought. It is carried out through the use of monthly precipitation forecasts from the European Centre for Medium-range Weather Forecasts (ECMWF). However, there continues to be little scientific and political attention that explicitly focuses on the early warning of agricultural droughts.

Overall, the EU policy approach for agriculture is mainly through effective adaptation measures with a range of technological solutions and adjustments for farming activities (EC, 2015). At a farm-level, short and mid-term solutions are to adapt the time of farm operations, select climate-resilient crops, improve the effectiveness of pest and disease control and water conservation strategies. In addition, sectorial plans seek to identify vulnerable areas, build adaptive capacity through training and awareness while supporting the agricultural research and experimental production. Finally, agriculture can also play an important role on mitigating climate change by diminishing its greenhouse gas emissions besides contributing to carbon sequestration.

Conclusion

Abiotic stress includes the list of environmental constraints (e.g. drought, nutrient limitations, heat, and salinity) which reduce grain yield of annual crops worldwide (Mueller et al., 2012). These stresses can differ in duration and intensity, and can act simultaneously or sequentially (Loomis and Connor, 1996; Mooney et al., 1991; Sih et al., 1998). According Loomis and Connor (1996) the law of minimum (von Liebig, 1855; De Wit, 1992) is adequate for capturing the effect of multiple stresses in a short time interval (e.g., hours), with growth affected by one stress at a time. In longer periods, nevertheless, the final effect of different factors which disturb plant growth does not represent the effect of any individual factor but of their interaction. This interaction effect is normally of multiplicative and not of additive nature once expressed in relative terms respect to potential conditions that maximize grain yield (Sadras, 2005). Furthermore, multiplicative effects are typically the result of stresses that take place sequentially along the cycle (Sadras, 2005; Rossini et al., 2016).

So, for example, drought and heat stress are two different abiotic stress conditions that occur mostly in the field simultaneously (Heyne and Brunson, 1940; Craufurd and Peacock, 1993; Jiang and Huang, 2001). Several studies have examined the effects of a combination of drought and heat stress on the growth and productivity for example of wheat, maize, barley, sorghum and different grasses. It was found that a combination of drought and heat stress had

a significantly greater detrimental effect on the growth and productivity of these plants and crops compared with each of the different stresses applied individually (e.g. Savage and Jacobson, 1935; Heyne and Brunson, 1940; Craufurd and Peacock, 1993; Savin and Nicolas, 1996; Perdomo et al., 1996; Jagtap et al., 1998; Jiang and Huang, 2001; Mohammadi et al., 2004; Wang and Huang, 2004; Mittler, 2006; Khurana et al., 2015, Vignjevic et al, 2015; Alghabari et al., 2016, Rossini et al., 2016). These studies make clear, that the response of plants to a combination of two different abiotic stresses is unique and cannot be directly extrapolated from the response of plants to each of the different stresses applied individually. Still limited physiological, molecular and metabolic studies performed with plants that were simultaneously subjected to two or more different abiotic stresses were done (Mitter, 2006).

3. NaCl and heavy metal impact on crops

In the present era of fast-occurring climatic changes, that have a big impact on all spheres of mankind, it is very important to maintain the optimal level of agricultural production, especially in developing countries of Europe, but also to preserve and even improve soil quality worldwide. Two of the most prominent problems due to climate change and inconvenient agricultural practice are soil salinisation and heavy metal accumulation.

The majority of crops cultivated in Europe are glycophytes and they are adversely affected by saline conditions. As a consequence, crops endure salt stress, defined as a condition where the plant is unable to express its full genetic potential for growth, development and production, as the soil salinity exceeds critical levels (Läuchli and Epstein, 1990). While naturally saline conditions exist in certain parts of Europe, mostly the soil salt content is resulting from human activities, such as inappropriate irrigation practice, usage of salt rich irrigation water and poor drainage conditions. Most of salt stress is caused by NaCl (Levitt, 1980). Excess salt levels are believed to affect around 3.8 million ha in Europe (EEA, 1995). While naturally saline soils occur in Spain, Hungary, Greece and Bulgaria, problems of artificially induced salinisation is affecting significant parts of Italy, Romania and Serbia (Szabolcs, 1974).

One of the implications of human induced disturbance of natural cycles is accumulation of heavy metals (HMs). They belong to group of nonbiodegradable inorganic chemical constituents with the atomic mass over 20 and density higher than 5 g cm^{-3} that may have adverse impact on plants. The group of elements with mostly unproven plant necessity in biological and physiological reactions consists of: As, Cd, Cr, Pb, Ni, Ag and Hg (Rascio and Navari-Izzo, 2011). Both underground and aboveground surfaces of plants are able to

take up HMs. The presence of these HMs in excess amounts may lead to reduction and inhibition of growth and physiological processes in crops (Zengin and Munzuroglu, 2005). According to Tóth et al. (2015), 6.24% or 137.000km² of agricultural land in Europe is contaminated with HMs.

To establish the strategies of crop management in sense of mitigating the stress effect, it is very important to overview the main impacts of these two types of stresses on crop growth, physiologic reactions and production.

Main NaCl and HM effects on crops

Both salt and HM stress may have impact on metabolic and physiological processes in plants, such as growth, photosynthesis, mineral nutrition and lipid metabolism.

Plant growth

One of the most common impacts of salt is clear stunning of plants (Hernandez et al., 1995). The first response to higher salt concentrations is reduction in rate of leaf surface expansion and changed dry mass/leaf area ratio. After Daničić et al. (2016), dry mass/leaf area ratio was already significantly decreased after exposure of safflower to 25.01 and 50 mM NaCl L⁻¹, relative to control. Also, Lazić et al. (2017)-unpublished results showed that leaf area per plant decreased in 3 species (*Sinapsis nigra*, *Sinapsis alba* and *Brassica napus*) after their subjection to 50 and 100 mM NaCl L⁻¹. Increasing salinity is often accompanied by significant reductions in number of leaves per plant (Mohamed et al., 1998). Relatively low NaCl concentrations in nutrient solutions (25.01 and 50 mM L⁻¹) adversely affected number of leaves per plant in safflower (Daničić et al., 2016). After Hernandez et al. (1995), salt stress also results in decrease of fresh and dry weight of plant tissues. To support this, Maksimović et al. (2010) reported that continuous presence of 0.6 and 1.2 g NaCl L⁻¹ declined leaf DW of pea (*Pisum sativum* L.), with respect to control. Daničić et al. (2017)-unpublished results obtained similar results in safflower.

Even though plants require certain HMs for their optimal growth, excess amounts of these HMs may become toxic to them (Djingova and Kuleff, 2000). The effect of HMs on plant growth may vary according to the particular HM involved. For metals such as Pb, Cd, Hg and As, adverse effects were recorded at very low concentrations in growth medium. Kibra (2008) recorded significant reduction in height of rice plants cultivated on a soil contaminated with 1mg Hg kg⁻¹. Reduction in shoot and root growth in wheat was revealed when Cd in the soil solution was as low as 5 mg L⁻¹ (Ahmad et al., 2012). After Gani et al.

(2009) 5 species from *Brassicaceae* family significantly decreased fresh and dry weight when they were treated with 10^{-5} CdCl₂.

Water relations in plants

After Morales et al. (1998), water and osmotic potential are negatively affected by the increase in salinity level, while the turgor pressure increases. As reported by Aziz and Khan (2001), leaf water and osmotic potential and xylem tension increased following increase in NaCl level in growth medium. Relatively low NaCl concentrations (8.33, 25.01 and 50 mM L⁻¹) adversely affected transpiration intensity in safflower, with respect to control (Daničić et al., 2016). In addition, Lazić et al. (2017)-unpublished results, pointed out significant decrease in transpiration intensity in oilseed rape, when it was subjected to higher NaCl concentrations (50 and 100 mM L⁻¹), relative to control.

In the presence of HMs, leaf area and root area decrease. This decline in root area affects the uptake of water. In the presence of 10^{-5} NiSO₄, increased transpiration intensity in 5 *Brassica* species was reported by Gani et al. (2009). After Petrović et al. (1992), some HMs affected density of the leaf stomata. It was increased in the presence of HMs, but their sizes decreased. After Gani et al. (2009), addition of Ni to growth medium of 5 *Brassica* species decreased leaf area and increased transpiration coefficient. Kastori et al. (1996) pointed out that the relative water content in plant tissue decreases along with the content of HMs.

Photosynthesis and chlorophyll content

Chlorophyll and carotenoid content in leaves generally decrease under both types of stress (NaCl and HMs). As reported by Daničić et al. (2016), concentrations of Chl a and b and carotenoids decreased in presence of low NaCl concentrations (25 and 50 mM l⁻¹) in safflower. Similar results were obtained by Putnik-Delić et al. (2016) in coriander. In addition, Lazić et al. (2017)-unpublished results reported significant decrease in total chlorophyll and carotenoid content in *Brassica nigra* subjected to 50 and 100 mM NaCl L⁻¹. Effect of HMs on the chlorophyll content depends mostly on concentration of HM. Gani et al. (2009) reported significant drop in Chl a and b and carotenoid content in all 5 *Brassica* species treated with Cd. This decline was more than several times higher relative to Ni application. According to Griffiths (1975), the reason for decreased Chl content is their reduced biosynthesis. However, HMs also stimulate Chls degradation.

Peroxidation of membrane lipids is well-known consequence of oxidative stress in plants caused by presence of salt or HMs. The content of malonyl-dialdehyde- MDA is indicator of lipid peroxidation (Ashraf and Ali, 2008). Application of 25.01 mM NaCl L⁻¹ significantly increased MDA content in coriander leaves Putnik-Delić et al. (2016). Similar results were obtained upon oilseed rape.

After Cd treatment, MDA content in wheat (*Triticum aestivum* L.) shoot and root was significantly increased (Luo, 1999).

Mineral nutrition

After Gorham et al. (2007), the interaction between ions, with respect to their uptake and accumulation in stress conditions is very complex. In general, concentrations of K⁺, P, Ca²⁺ and Mg²⁺ in plant tissue decrease in presence of excess NaCl, mostly due to antagonistic relationship to Na⁺ which is dominant in saline conditions. To support this, Maksimović et al. (2010) reported a significant drop in K⁺, P and Ca²⁺ concentration in different tissues of pea (*Pisum sativum* L.) subjected to low NaCl concentrations. In addition, Daničić et al. (2017)-unpublished results obtained similar trend in safflower. Availability of micronutrients mostly depends on the pH and pE of soil solution, but also on the nature of binding with the organic and inorganic particles of the soil. Most studies show that in saline conditions the availability of micronutrients is very low or variable (Page et al., 1990).

Decline in nutrient uptake depends on concentration of HMs, plant species, specific genotype etc. (Yang et al., 1996). Applied Ni in concentration of 10⁻³ and 10⁻⁵ M did not significantly affected K⁺ concentration in wheat and maize (Ilin, 1997). Opposite to this, Gani et al. (2009) reported significant decrease in K⁺ content in *Brassica napus* when it was subjected to 10⁻⁵ M of NiSO₄. The very same experiment showed that P and Mg²⁺ concentrations increased while Ca²⁺ concentration decreased in the presence of 10⁻⁵ M Ni.

Concentrations of micronutrients varied under Ni conditions. Wang (2009) reported that Cd significantly inhibited maize from absorbing N, P and Zn and enhanced Ca²⁺ uptake. The effects on absorption of P, S Ca²⁺, Fe and Zn are more complicated and related to plant species, type of stress, pH and presence of other nutrients.

Mitigation strategies of plants induced by salt and HM stress

To cope with different kinds of stress conditions, plants respond with physiological and biochemical changes which aim the maintainance of basic metabolic processes. Sometimes these strategies may enhance tolerance of plants to abiotic stress.

Activation of enzymes

Salt and HM stress cause adverse effects on a wide variety of metabolic activities of plants, which result in oxidative stress because of formation of reactive oxygen species such as superoxides, hydroxy and peroxy radicals (ROS). ROS cause membrane disfunction and cell death (Bohnert and Jensen, 1996).

Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are important enzymes that help plants to mitigate oxidative stress. The harmonious interactions of the three enzymes, keep the ROS level in plant tissues low, preventing injury of plant cell structure. Activity of ascorbat peroxidase increased in wheat cultivated under saline conditions (Hernandez et al., 2000). SOD activity in plant leaves of barley was increased following salinity level (Liang et al., 1999). Lazić et al. (2017)-unpublished resuts showed enhanced concentration of CAT in leaves of *Sinapsis nigra* L. subjected to 50 and 100 mM NaCl L⁻¹. Similar response was determined in case of guaiacol peroxidase.

The activities of SOD, POD and CAT are usually enhanced by HM presence (Cd, Cr⁶⁺, Hg, Ni, Pb and Fe) in low concentrations (Dong, 1999), but as the concentration of HMs increase, the activity of enzymes decrease. The activity of SOD in shoot and root of wheat exposed to Pb significantly enhanced and activity of CAT was also improved. After Kastori et al. (1996) in the presence of HMs, activity of nitrate reductase declines. This was supported by findings of Gani et al. (2009), when 5 *Brassica* genotypes were subjected to Cd.

Synthesis of proteins and aminoacids

Many sensitive plants accumulate free proline as a nontoxic and protective substance under salt and HM stress (Lee and Liu, 1999). Maggio et al. (2002) are of a opinion that proline may act as signaling molecule able to activate multiple responses which participate in adaptation processes of plants. Free proline concentration significantly increased in fresh leaves of safflower after its exposure to relatively low NaCl concentrations (25 and 50 mM L⁻¹). In addition, Lazić et al. (2017)-unpublished results indicate that under 50 and 100 mmol

NaCl L⁻¹, free proline accumulated significantly higher in 3 treated species (*S. nigra*, *S. alba* and *B. napus*), relative to control.

Under low Cd concentrations ($\leq 20 \text{ mg L}^{-1}$), content of free proline increased in wheat. As an important substance for mitigation of HM stress, proline content increased in 5 *Brassica* genotypes, after they were subjected to Ni and Cd (Gani et al., 2009).

Ascorbic acid

Phenolic substances and ascorbic acid (vitamine C) are secondary metabolites that act as strong natural antioxidants (Balasundram et al., 2006). They inhibit lipid autooxidation and play important role in scavenging ROS, which makes them essential antioxidants (Namiki, 1990). Fresh leaves of coriander, subjected to 50 mM NaCl L⁻¹ showed significant increase in vitamine C concentration (Putnik-Delić et al., 2016).

Silicon (Si)

Si is the second most abundant element in the Earth's crust, covering 27.7% of the lithosphere. Si is considered as „quasi- essential“, or nonessential element for plants, because most of plant species can complete their life cycle without it (Arnon and Stout, 1939). Under abiotic stress conditions, Si application results in alleviation of stress and enhancement of plant growth (Soundararajan et al., 2014). Lazić et al. (2017)-unpublished results showed that leaf area per plant of *Brassica napus* increased significantly after addition of Si to 50 mM NaCl L⁻¹. Also, fresh weight of stem and roots per plant of *Sinapsis alba* and *Brassica napus* increased after addition of Si to 50 mM NaCl L⁻¹. The concentration of guaiacol peroxidase in roots of *Sinapsis nigra* increased, after its exposure to NaCl and Si conditions, as compared to only NaCl -applied treatment (Lazić et al., 2017)-unpublished results.

Si significantly improved growth and biomass of rice plants and reduced toxic effects of Cd after different stress periods. There was also reduction of lipid peroxidation in plant tissues (Kim et al., 2014). Langer et al. (2009) previously found that HMs applied to rice plants caused weak root pattern and morphology, while addition of Si ameliorated this impact.

Conclusion

The impact of global climate change on crop production has emerged as a major research priority during the past decade. Understanding abiotic stress factors such as NaCl and heavy

metal, in combination with high yield in plants is of a paramount importance to counter climate change related adverse effects on the productivity of crops.

4. N deficiency stress on crop

Fundamentally, plants require energy (light), water, carbon and mineral nutrients for growth. Abiotic stress is defined as environmental conditions that reduce growth and yield below optimum levels. Nitrogen (N) represent one of the main factor of soil fertility as the first element required by the plants for their growth and life. N into the plants is biologically combined with carbon (C), hydrogen (H), oxygen (O), and sulphur (S) to create amino acids, which are the building blocks of proteins. Amino acids are used in forming protoplasm, the site for cell division and thus for plant growth and development. Furthermore, N is a major part of the chlorophyll molecule and is therefore necessary for photosynthesis and is a necessary component of several vitamins. Despite the fact that N represent 78% of global atmosphere only a small fraction is available for plants. It represents 1-3.5 % of dry matter of living organisms (Radaelli and Calamai, 2001) and, through the cultivation activities, its rate into the soil decreases year by year due to different factors as crop uptake, soil erosion, leaching and volatilization. Soil contains commonly from 0.1% to 0.6% of N in the first 15 cm that it means 2.000 to 12.000 kg N ha⁻¹ depending on the soil type and the rate of organic matter (Cameron, 2013).

Nowadays, human activities are very impactful at the global level and agriculture is not an exception. It is largely known that the progress of production processes of our society may affect the global scenarios. Projections of world population increasing affirm that in 2100 humans will reach 10 billion of people. This fact requires an increasing in food production from agricultural sector related to cultivation strategies that ensure to maintain soil fertility with a reduction of impacts on the environment. In this way, one of the most impactful sector of agriculture is the fertilization. Fertilizers application, especially N, is a fundamental practice to obtain high crop production but from the other hand produce strong impact on the environment. Fertilization produces direct GHGs emissions in field by tractors and indirect GHGs emissions during the interaction with soil/plant system. However, maintaining of soil fertility is fundamental for the cultivation of crops. Spreading method and the type of fertilizer (organic or mineral) play a key role on the crop yields and on the environmental impact of fertilizers due to the losses that can be generated. Because of that, in the last decades a lot of attention is provided to found practices that ensure the highest efficiency

level in N-based fertilizers use. However, the need of high crop yield in addition to low efficient spreading methods can generate and overdose of distributed N with damages to the crops and the environment. This phenomenon is amplified by water that can dissolve and transport N with the risk of eutrophication in downstream areas.

Nitrogen deficiency effects on crops

Deficiency or excess of N in the soil can generate adverse effect for crop growth of crop and in some cases can result in a loss of yields. Nowadays, both situations can occur due to intensive agriculture that consumes many resources and produces high impact on the environment (soil erosion, leaching, emissions etc.).

When N content of the soil is too low for the growth of crops, fertilization is required to reach a satisfactory level on crop yield. N deficiency in crops can cause significant economic, environmental and yield damages.

Lack in chlorophyll and reduction of photosynthesis

Lack of chlorophyll with a reduction of photosynthesis and change in leaf color from green to yellow are the first symptoms that plants show due to N deficiency. N deficiency usually triggers and accelerates leaf senescence at the time of full-expanded leaves. Craft-Brander et al. (1998) found that N deficiency led to decline of proteins located in wheat leaves in the chloroplast, peroxisomes and cytosol. Therefore, lower photosynthetic capacity is due to reduced carboxylation efficiency (Huang et al., 2004). Degradation of photosynthetic pigments and proteins is the typical characteristic of leaf senescence and the deficiency of N produces this alteration in herbaceous crops (Huang et al., 2004 and Zhao et al., 2005) as in trees (Boussadia et al., 2010). Leaf discoloration starts from the base of the plants (oldest leaves) to the top (youngest leaves), due to the transport of N from the older to the youngest tissues.

Plant growth

As a consequence of the reduction of photosynthetic activity plants show reduced accumulation of dry matter (DM). This is confirmed by Boussadia et al. (2010) and Zhao et al. (2005) that discovered that N deficiency decreases leaf area (LA). A stunted growth with reduced stems development, and roots with scarce ramifications and low depth may occur due to a reduction in cell division following the lack of N. Evans (1982) affirmed that N deficiency strongly affect growth of leaves in the first phases of plants. This phenomenon is

emphasized for C3 plants due to a stomata closure and difficult in carbon dioxide (CO₂) assimilation (Boussadia et al., 2010 and Ciompi et al., 1996). Moreover, root system is strongly affected by N deficiency and the resultant situation is a reduced expansion of roots with an imbalance between epigeal and hypogeal part of plant.

Flowers, seeds and fruits

Flowering result in a scarce rate and a great number of them fall from the plants during the first days. Fruits anticipate ripening, size is low and, as flowers, fall from the plants after some days from ripening. Yield and quality are low due to a lack in proteins and carbohydrates. Seeds show reduced size, protein content is low and, in general, their quality decreases with a reduced germination potential.

From the other hand, an excess of N into the soil can be also dangerous for plants and for yields.

Elongation of biological cycle of plants

N is abundant in the young tissues of the plants where metabolic activities are concentrated. An excess of N can induce the plant in elongation of the biological cycle extending the vegetative phase. Results are increasing in stems length that became less resistant and increase the risk of breaking the stems and branches, in trees, or plant lodging, in herbaceous plants. The main rate of plant energy is redirected on foliage proliferation, so plants may not even produce their necessary reproductive organs during the growing season. Extension of vegetative phase induces an increase of water consumption, as increase in evapotranspiration, due to the great metabolic activities. Moreover, the elongation of vegetative phase gives cellular wall slightly thickened, lignification is reduced or absent. This situation makes plants more susceptible to pests and low temperatures.

Factors affecting N dynamics

Emissions. If from one hand N-fertilization provide the nutrition for crops and ensure satisfactory yields, from the other can represent a source of different impact that can negatively affect the environment. This way, emissions of GHGs represent the main impact of fertilization with N volatile compounds as nitrous oxide (N₂O) and ammonia (NH₃). N₂O is a greenhouse gas and his Global Warming Potential (GWP) is around 290-300 times more than CO₂ (IPCC). N₂O is mainly produced in anaerobic conditions from nitrate (NO₃⁻) as a product of de-nitrification and in a small part in aerobic conditions as by-product of

nitrification. In the last decade there was a large scientific effort to understand these processes and, in particular, to develop methods to reduce N₂O emissions. NH₃ is a precursor of N₂O and emissions depend on fertilizer composition, soil moisture and atmospheric temperature. If ammonium (NH₄⁺) is not quickly absorbed by plants or fixed into the soil, NH₃ volatilization can occur.

Essentially, factors that mainly affect N emissions are: soil moisture content that affects the aeration status of the soil and this affects de-nitrification; soil texture that play a key role directly affecting drainage potential and O₂ level into the soil; composition of N-based fertilizer.

Eutrophication. Eutrophication is a phenomenon that consists of the enrichment of water with nutrients, mainly N, followed by an excess of the element released into the water. Eutrophication induces the proliferation of plants and algae and, due to the biomass load, may result in oxygen depletion of the water body. Frequently, eutrophication represent a problem when low efficiency irrigation strategies are used in correspondence to abundant fertilization. The understanding of the mentioned process and the planning of irrigation-fertilization represent the first mitigation strategy.

Water content in the soil

Leaching. Leaching is defined as the removal of N (and other nutrients) from the soil by the action of water such as rain or irrigation. Leaching represents the first factor that affects the translocation of N in the deep layers of the soil and can contribute on the contamination of groundwater. Therefore, N leaching from soil not only represents a loss of soil fertility but also represents a threat to the wider environment and to human health (Cameron et al., 2013). NO₃⁻ represents main resource for crop N-nutrition but is also affected by leaching that can strongly reduce the fertility of soil with nutritional stress of the crops. Especially in sandy soils, that show a low adsorption potential, leaching may represent an important issue for the soil fertility. Considering the previous statements it is possible to affirm that soil texture, irrigation rate and the kind of fertilizer (rich in NO₃⁻ or in NH₄⁺) are fundamental aspect of N managing and plant health.

De-nitrification. During the process of de-nitrification nitrate is reduced with production on molecular nitrogen (N₂) and N₂O. The main condition for de-nitrification is anaerobic, mainly due to a saturation of soil with water. Temperature is also affecting de-nitrification because microorganisms that perform the process need more than 20°C, under this level de-

nitrification is negligible and at 2°C it terminate. Moreover, pH is a factor that affects the process. Microorganisms need pH level between 5 to 8, and under this range they aren't able to proceed the process.

Fundamentally, at the normal agricultural conditions de- nitrification rarely occurs. During summer main cause of it is lack of water and during the winter low temperature reduce the risk of de- nitrification. However, field conditions monitoring is an efficient strategy to reduce the risk of de- nitrification and N emission.

Temperature. Temperature plays a key role on the dynamics of N into the soil because it affects a wide range of microbiological processes. Fundamentally, temperature affects the microbiological population into the soil that may proceed nitrification and de- nitrification. Moreover, NH₃ emissions are encouraged by high temperature that provides the optimum habitat for bacteria responsible of the process.

Strategies to mitigate N deficiency in plants

Mitigation strategies that can reduce N stress on crops are represented by the application of the good agronomic practices. In particular the main strategies consist of:

- High efficiency fertilization. Spreading of fertilizers is one of the main factors that affects N availability for the plants and reduce emission losses. Fertilizers that show high rate of NO₃⁻ are sensitive to leaching. Spreading of fertilizers has to be performed in the high-nutrient request period of the crop and when soil moisture content allows oxygen exchange from soil to the atmosphere. Regarding to NH₄⁺-based fertilizers, that are sensitive to emissions losses, the incorporation into the soil of fertilizer represent the main strategy to reduce losses and N stress on plants. As affirmed by Wulf et al. (2002), the more the fertilizer is incorporated into the soil the less N is lost.
- Control of water supply of the field. As mentioned above, water represents an important factor that affects N availability in the soil. As general strategy, it is fundamental to find the more efficient strategies that reduce losses and allow creation of right water/oxygen rate into the soil (etc. drip irrigation).
- Crop rotation. Crop rotation allows creation of healthy environment that show a good structure of soil with the right rate of porous, right amount of nutrients (especially after nitrogen fixing plants) and a wide microbiological population.

- Soil erosion control. Soil erosion represents a source of N (and other nutrients) losses. The application of the good agronomic practices as control of water supply, crop rotation and soil management reduces soil erosion risk.
- Climate monitoring. Monitoring of climate, especially temperature and precipitation, allow maintainance of soil in the best condition for plant growth. Due to climate change precipitation are changing with low number of rainy days but with higher intensity that may produce agricultural disaster such as loss of fertility.

Combination of N/ water deficiency effect on crops

The combination of water and N deficiency may produce relevant damages in plants. In particular, water deficiency reduces stem growth and root expansion. But from the other hand N deficiency may produce same symptoms that are emphasized with a great reduction of plant vitality. In this situation plant show a stunted growth because they are deficient in N which is the fundamental constituent of a large number of primary components (etc. proteins) and also deficient in water. Lack of water stops evapotranspiration and, as a consequence, a large amount of physiological processes.

However, as demonstrated by Eghball et al (1991), water stress shows greater damages than N deficiency. In particular, in absence of water plants show a lower rate of growth of roots than in absence of N. If the situation persists over time, the plant goes dead in the short term.

Combination of N/salinity deficiency on crops

Salinity stress decreases plant growth due to nutritional deficiencies shown by N decreasing root to stem ratio of dry weight. N compounds synthesis is lowered through the inhibition of nitrate reduction and ammonium assimilation. This is explained by the low nitrate availability and possible inactivation activities by Na^+ and Cl^- ions. The high salt concentrations enhance proteolytic activity and ammonium production (Silveira et al., 2001; Debouba et al., 2006).

Conclusion

N availability in the soil represents one of the main factors that have to be considered for crop production. Considering climate change and world human population growth the understanding of the abiotic stress for plants as N dynamics represents a priority. Research plays a key role to define agricultural sustainable management strategies that allow to produce food for world population reducing the impacts on climate change phenomenon.

Overall conclusion

Over the last decade, climate change has been recognized as an additional factor which will have a significant impact on agricultural production. Based on literature sources, in the absence of adequate response strategies of crops to long-term climate change consequences, as well as to climate variability diverse and specific impacts will become more apparent. Some of those impacts are expected to be adverse. At times, impacts will be slow to unfold enabling local farmers and governments to respond. Impacts of climate variability and change, on the agricultural sector are projected to steadily manifest directly from affecting deterioration of abiotic factors to plants. Climate change is expected to result in long term water and nutrient shortages as well as worsening soil conditions, causing drought and salinity. Vulnerable areas (such as some Europe regions) may experience losses in agricultural productivity, primarily due to reductions in crop yields. Early estimates suggest 14-16 percent losses in developing countries of Europe due to climate change- induced effects on crops.

It is unavoidable for producers to experience long term consequences of climate variation, but in a field of agriculture, in terms of declining the stress effect on crops, science has already given many crucial answers. By being familiar with plant physiology, strategies of adaptation and mitigation of new conditions, it is possible, at least in part, to alleviate stressful impacts on crops.

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