Environmental and Experimental Botany 92 (2013) 134–143

Contents lists available at SciVerse ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Symbiotic nitrogen fixation in legumes: Perspectives for saline agriculture

Bas Bruning*, Jelte Rozema

Systems Ecology, Department of Ecological Science, Faculty of Earth and Life Sciences, VU University Amsterdam, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 23 March 2012
Received in revised form 24 August 2012
Accepted 3 September 2012

Keywords:
Saline agriculture
Legumes
Nitrogen fixation
Salt tolerance
Salt sensitivity

ABSTRACT

Saline agriculture provides a solution for at least two environmental and social problems. It allows us to return to agricultural production areas that have been lost as a consequence of salinization and it can save valuable fresh water by using brackish or salt water to irrigate arable lands. Sea water contains (micro) nutrients that can provide the additional benefit of a reduced need of fertilization in saline agriculture. However, nitrogen is only present in very low quantities in seawater. A salt tolerant nitrogen-fixing legume used as a vegetable crop, fodder or green manure could increase the availability of soil nitrogen as well as the sustainability of saline agriculture while minimizing the application of inorganic fertilizer. Besides the use of salt-tolerant legumes as green manure, such species could also be useful in salinized areas as fodder and/or human food.

In this review, we assess the feasibility of the use of legumes in saline agriculture. Most legumes are sensitive to salinity, as is the process of nitrogen fixation by microorganisms in the nodules of the legumes. First, we identify different steps in nodulation and their respective sensitivity to salinity. We will then look at the sensitivity of the process of nitrogen fixation in various crop and non-crop legumes, differing in their tolerance to salinity. We will also look into the differential response of nitrogen fixation and biomass production to salinity. Finally, a list of salt tolerant legumes is presented (derived from the HALOPH database). We then evaluate the applicability and perspective of salt tolerant legumes in saline agriculture considering the diversity in growth forms, ecotypes and economic uses.

1. Introduction

Salinity leads to several physiological stresses in plants and consequently few plants can tolerate significant salinity levels in their root medium for any length of time. Of all the worlds' species, about 1% are considered halophytes (Rozema and Flowers, 2008), which are defined by Flowers and Colmer (2008) as able to complete their lifecycles under saline conditions corresponding to at least 200 mM of NaCl in the root medium. Non-halophytes are called glycophytes. The negative effects of salt on plant growth and development has been shown by numerous experiments, reviewed several times and has been discussed in length in this special issue. The negative effects of salt on plant growth are a considerable problem for agriculture and thus the world food production.

Salinization has been an issue in agriculture for thousands of years (Jacobsen and Adams, 1958). Currently it is causing problems in many parts of the world (especially in arid and semi arid regions, Manchanda and Garg, 2008); and it is predicted to get worse under climate change conditions with increasing weather extremes and rising seawater levels (Rozema and Flowers, 2008). Worldwide, up to 20% of arable land surface is salt affected (FAO, 2002). Soil salinity relates to the build-up of salts in soil and can be both natural and anthropogenic. A soil is considered saline if the electric conductivity of a saturated paste (equivalent to the available salts in the soil pore water) of that soil is over 4 dS/m (equivalent to ±40 mM NaCl, which is roughly equivalent to $40/550 \times 100 = 7\%$ of seawater salinity).

Notwithstanding the sensitivity of many plants to salinity, some plants can survive and grow vigorously under saline conditions (Rozema and Schat, 2012). The use of such plants would be extremely helpful not only to reclaim salinized areas, but also because it would allow us to use brackish or slat water for irrigation in agriculture.

The use of brackish and saline water for irrigation is related to the scarcity of fresh water in the world, especially in areas that receive little annual rainfall. About 1% of the world's water is fresh (>0.05% dissolved salts), about 97% of the water is seawater (<3% of dissolved salts; Rozema and Flowers, 2008) and the remainder is of intermediate salinity. Humans use large quantities of fresh water (and the demand increases faster than the human population grows) for a variety of activities including industry (20%), domestic use (10%) and, most notably, agriculture which accounts for around 70% of global fresh water consumption.

The salinity problems and the scarcity of fresh water point to a solution via the use of salt-tolerant plants in agricultural production. Few crop plants can tolerate even moderate levels of salinity however. Attempts to improve the salinity tolerance

* Corresponding author. Tel.: +31 20 5982737.
E-mail address: b.bruning@vu.nl (B. Bruning).

0098-8472/$ – see front matter
http://dx.doi.org/10.1016/j.envexpbot.2012.09.001

of conventional crops have not been successful so far. This is because tolerance to salinity is a complex trait (Delgado et al., 1994), involving multiple genes and having evolved multiple times independently among different lineages (Flowers et al., 2010a), leading to different mechanisms of salinity tolerance. A different promising strategy is to focus on the de novo domestication of halophytes.

Seawater contains many elements required for plant growth. This would reduce the need for fertilization of arable lands under saline irrigation for many (micro) nutrients. However, the element plants require in the highest quantities, nitrogen (N), is present in sea water in very small quantities, and would therefore have to be supplemented. Conventional farming uses large quantities of fertilizer to supply crops with nitrogen but the process of artificial nitrogen fixation, the Haber–Bosch process, occurs under high temperatures and pressures and thus requires high energy inputs, rendering it unsustainable in the long term (Peoples et al., 1995).

In natural ecosystems, there are several inputs of soil nitrogen. Even though our atmosphere consists of nearly 80% of N_2, it is the limiting element in most ecosystems (Vitousek and Howarth, 1991). Bio-available nitrogen can enter an ecosystem via atmospheric nitrogen deposition, mostly in the form of ammonium and nitrogen oxides (NO_x). The source of this nitrogen is mostly anthropogenic for these compounds, but nitrogen oxides are also formed by lightning (about 10% of nitrogen deposition on the land). However, the largest input of nitrogen comes from nitrogen fixed by microorganisms, called diazotrophs (Zahran, 1999). These can be either free living or associated to plants from, mainly, the family of the Fabaceae. Nitrogen input from legumes can be a sustainable source of nitrogen in agricultural systems (Peoples et al., 1995).

Therefore, in this review we will focus on the (potential) use of legumes in saline agriculture, as crops, fodder, forage or other economically viable uses. However, the Fabaceae plant family seems to be particularly sensitive to salinity (Maas and Hoffman, 1977). First, we will describe some general characteristics of legumes and the use of nitrogen in agricultural systems. We will then review the potential of legumes as green manure in saline agriculture by looking at some examples and assess the salt tolerance of some legume species, as well as the sensitivity of the nitrogen fixation process to salinity. We will also look closer into the often made statement that biomass production is less sensitive than nitrogen fixation to salinity. Then a list of salt tolerant legumes is presented (from the HALOPH database). We will then also discuss other legumes, tolerant to salinity but for undetermined reasons not listed in the HALOPH database. Finally, we make some recommendations about how to proceed with the development of a sustainable saline agricultural system.

When salt concentrations are mentioned in this review, the unit of the original publication is used, except in graphs as in Figs. 2 and 3. Some caution is advised interpreting these data since salinity tolerance depends on various (experimental) conditions (Maas and Hoffman, 1977). For this reason we mainly mention salinity tolerance categories: salt sensitive (80% of biomass production as compared to control at ~3 dS/m (equivalent to about 30 mM NaCl), moderately sensitive (80% of biomass production at ~6 dS/m; 60 mM NaCl), moderately tolerant (80% of biomass production at ~11 dS/m; 110 mM NaCl) and tolerant (80% of biomass production at ~16 dS/m; 160 mM NaCl) (Maas and Hoffman, 1977). 16 dS/m equates to about 30% of seawater salinity. The slope of the line that describes the relationship between salinity tolerance and salt concentration becomes steeper with increasing salinity tolerance (i.e. less biomass reduction takes place for more tolerant plants as compared to less tolerant plants with equal size steps of increasing salinity concentrations).

1.1. Legumes and nitrogen fixation

Legumes (Fabaceae) are a family of dicotyledoneous plants, 88% of which (Defaria et al., 1989) form a symbiosis in their root systems (sometimes on their shoots; Loureiro et al., 1995) with nitrogen fixing microbes (diazotrophs; in association with legumes collectively called rhizobia in this paper) in specialized organs called nodules. Legumes are important food crops all over the world (e.g. Dita et al., 2006), in part because of their high nitrogen content which in turn is a result of the symbiosis with the rhizobia. Legumes are also widely used as green manure since the beginning of agriculture, although this has diminished since industrially produced fertilizer became available (Zahran, 1999). Green manure adds nitrogen to the soil and improves soil quality by increasing the organic matter content of the soil. Of the plants' total nitrogen content, on average 70–80% (range: 6–91) is fixed by the symbiotic rhizobia (Peoples et al., 1995). The remaining plant nitrogen is taken up by the root system as in other plants. The increase of yield after growing green manure is most pronounced in the first season after growing the legumes (Sullivan, 2003).

The symbiotic microorganisms in the root nodules, the rhizobia, can take up gaseous dinitrogen (N_2) from the air and 'fix' the nitrogen into molecules (ammonia or amino acids) that can be assimilated by the plant. In return, the plant provides the rhizobia with a carbon source in the form of dicarboxylic acids (i.e. Soussi et al., 1999). The enzyme responsible for the nitrogen fixation, nitrogenase, is irreversibly damaged when exposed to oxygen. The plant produces leghemoglobin, a protein related to human hemoglobin to provide the rhizobia in the nodules with oxygen, often giving functional nodules a pink color.

The symbiosis first appeared around 58 million years ago when the *Papilionoideae* (a subfamily of the *Fabaceae*) underwent genome duplication (Young et al., 2011). Interestingly, the genes involved in the signaling seem to be originally involved in the symbiotic relationship with mycorrhiza. Thus, it seems that only through this whole genome duplication, genes became available that were free to fulfill this new function in the communication with rhizobia, thereby enabling the legumes to start this almost unique symbiotic relationship (Young et al., 2011).

1.2. Legumes in saline agriculture; nitrogen fixation capabilities

To evaluate the feasibility of the use of legumes as green manure in saline agriculture, we will first assess the nitrogen fixing capabilities of legumes under non-saline conditions. We will then compare this with the amount of fertilizer applied by farmers under the assumption that this reflects to some degree the demand of their crops. This is not always a valid assumption however. In many African countries, fertilizer use reflects the economic status of the respective countries more than the actual N requirement of the crop. As a result of these and other reasons, fertilizer use varies greatly among countries, from over 700 kg N/ha/yr in, for example, in Egypt, Costa Rica and Malaysia to less than 1 kg in many African countries, for example Niger, Namibia and Mozambique (FAO, 2008). However, for the purpose of comparison we assume 100 kg N/ha/yr as a reasonable approximation of fertilizer needs for most crops. For example, average nitrogen fertilizer use in the United States of America has been a little under 95 kg N/ha/yr (USDA ERS) between 1995 and 2005. Table 1 summarizes the amount of nitrogen some frequently used legumes can fix (after Phillips, 1980) which allows us to compare the addition of nitrogen via green manure with fertilizer use.

Most legumes in Table 1 are easily able to fix 100 kg N/ha/yr, and this figure fits well with findings in other studies; reported values of 200–300 kg N/ha/yr are no exceptions (Peoples et al., 1995). However, about 40–60% (Sullivan, 2003) of the nitrogen fixed is available