Purslane (Portulaca oleracea L.) an excellent source of

omega-3 and omega-6 fatty acids with abatement of risk

factors

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ABSTRACT

Wild plants are usually not consumed in daily diets, but represent a great source of nutrients for humans. Purslane, a plant known as a common weed, is known to have elevated concentration of fatty acids (ω -3 and ω -6), essential compounds for human health. The objective of this study was to determine how the nitrogen and photoperiod affected the concentration of fatty acids in purslane, as well as the concentration of oxalic acid (component with a slightly negative effect on human health). Nine accessions of purslane from Canada, Afghanistan, Saudi Arabia, Syria and Iran were studied in controlled environment growth chambers. Two ratios of nitrate: ammonium (50:50 and 25:75) fertilizers and two photoperiods (12 and 16 hours) were studied. Statistical analysis was performed using a factorial model with three levels. The leaves and stems of purslane plants that were grown under 12 hours photoperiod and 50:50 N0₃:NH₄⁺ had the highest concentrations of linoleic acid and α -linolenic acid, and the lowest concentration of oxalic acid. Among the accessions studied, an accession from Quebec epitomized the most viable selection for human consumption.

RÉSUMÉ

Les plantes sauvages, généralement non-consommés dans l'alimentation quotidienne, pourraient représenter une grande source d'éléments nutritifs pour les humains; le pourpier est une plante connue comme une mauvaise herbe, il est connu pour avoir une concentration élevée d'acides gras (α -3 and α -6), des composants essentiels pour la santé de l'Homme. L'objectif de cette étude était de déterminer comment l'azote et la photopériode affectent la concentration des acides gras du pourpier, ainsi que la concentration de l'acide oxalique (composant avec un effet légèrement négatif sur la santé de des humains). Neuf accessions de pourpier en provenance du Canada, d'Afghanistan, d'Arabie Saoudite, de Syrie et d'Iran ont été étudiées, des chambres de croissance ont été utilisées pour assurer un environnement de croissance maîtrisée. Deux ratios de nitrate d'ammonium: (50:50 et 25:75) ont été étudiés associés à deux photopériodes (12 et 16 heures). L'analyse statistique a été réalisée à l'aide d'un modèle factoriel à trois niveaux. Les résultats indiquent que les plantes cultivées en dessous de 12 heures de photopériode et 50:50 N03: NH4 + ont eu les plus grandes valeurs de concentration de l'acide linoléique et l'acide α -linolénique et la plus faible concentration de l'acide oxalique, en terme de feuilles et de tiges de pourpier. Parmi toutes les accessions étudiées, l'accession du Québec représentait la culture la plus viable pour la consommation humaine.

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PREFACE AND CONTRIBUTION OF AUTHORS

The research work presented here was performed by the candidate, Sara Marcela Eljach Mosquera, who executed all the experiments, collected the data, analyzed the data, and wrote all the chapters. Dr. Alan Watson, thesis supervisor, proposed the research question guiding the candidate in planning, designing and executing the experiments. He edited, corrected and reviewed all the information available in this document. Dr. Danielle Donnelly contributed in planning and executing some parts of this project. Dr. Kebba Sabally edited and reviewed the material and methods of this work.

1.0 INTRODUCTION

The recent exponential growth of the food industry has limited the intake of a large range of edible plants and restricted our possibility to have a varied and healthy diet. Plants offer a large variety and quantity of essential nutrients that the human body is incapable of making itself. Many plants contain several factors known to help protect the human body against common diseases, such as cancer and coronary heart disease. These protective factors are called nutraceutical (Fontana et al. 2006). Numerous studies indicate that many wild plants, not usually included in the current human diet, contain these nutraceutical factors, aiding the human body to avoid, protect and control several diseases common today. Plants, like purslane (*Portulaca oleracea* L.) and linseed (*Linum usitatissimum* L), have nutraceutical status and are recognized for their high nutritional content, particularly the presence of omega- 3-fatty acids in their leaves and stems (Omaraalwala et al. 1991, Palaniswamy at al. 2002).

Purslane has been described as a plant with an elevated nutraceutical level, providing a good source of nutrients to humans, as well as preventing several diseases. Despite being known as a weed, human consumption of purslane has been reported in different countries, mostly restricted to the Mediterranean area. Purslane is also recognized in North America for its high nutritional status (Simopoulos 1999, 2002).

Several studies have been made on purslane highlighting the elevated concentration of linoleic acid and α -linolenic acid present in its seeds, stems and leaves,

and the presence of antioxidants and oxalic acids the latter an anti-nutrient implicated in the development of kidney stones in susceptible individuals (Fontana et al 2006, Palaniswamy et al. 2001, 2002, 2004, Simopoulos 2002).

Two main options exist for modifying the concentration of fatty acid, as well as the concentration of oxalic acid: 1) the nitrate and ammonium ratio in the fertilizing formula and 2) the photoperiods. Several studies have demonstrated that these two characteristics play a fundamental role in the production of purslane with an increase quantity of fatty acids and a reduction in the concentration of oxalic acid (Palaniswamy et al. 2000, 2001).

Therefore, the purpose of this thesis was to understand how these two factors affect different accessions of purslane, both wild and agronomic; to attain the necessary knowledge to achieve marketable purslane offering a new edible weed with significant consumer health benefits; to define the viability of producing quality *Portulaca oleracea* for the fresh market in Quebec, and to offer a leafy vegetable with a great source of fatty acids (linoleic acid and α -linolenic acid) and low concentration of oxalic acid. This common weed may provide niche marketing opportunities for entrepreneurial growers in Quebec.

Specific objectives were: 1) to evaluate the vegetable quality of wild and agronomic accessions of purslane with respect to their yield, fatty acid and oxalic acid concentrations, 2) to compare the effect of two nitrogen (N) levels delivered as NO_{3⁻} and

NH₄⁺ in ratios of 50:50 and 25:75 on the yield, oxalic acid and fatty acid content of different accessions of purslane, and 3) to compare the effect of 12 and 16 hours/day photoperiods on the yield, oxalic acid and fatty acid content of different accessions of purslane. The accessions used in this work represented different geographical regions; Iran, Saudi Arabia, Afghanistan, Syria and Canada (3 local accessions) are the countries where the plants were collected; also two agronomic varieties, 'Tall green' and 'Golden', were used.

It can be hypothesised that: 1) the yield and fatty acid concentration will be higher and the oxalic acid will be lower in the wild purslane compared to the horticultural varieties, 2) the N0₃:NH₄+ ratio 25:75 will offer the yield with the highest fatty acid and lowest oxalic acid concentration; and that 3) the plants expose to 16 hours will produce higher yield than plants grown under 12 hours light photoperiod.

2.0 LITERATURE REVIEW

2.1 Purslane characteristics and description

Purslane belongs to the Portulacaceae family. It is an annual, succulent, prostrate herb, with thick tap root and many fibrous secondary roots (Figure 1). Its prostate growth habitat makes a mat up 60 cm in diameter per plant. Stems are reddish, glabrous, succulent, and fleshy and the toothless leaves are also succulent. The flowers are small and yellow-petaled. The spherical, many-seeded pods (globular pointed capsule), open with a circular lid (Miyanishi and Cavers 1980).

The purslane growing season is limited to the hottest months of the year in North America (June to September). The seed requires high temperatures for germination, which is optimal above 30 °C and extremely poor below 24°C. Plants are sensitive to cold and are killed by chilling temperatures. However, purslane seed can survive well in areas where winter temperatures are below -30°C and areas where the temperature reaches 50°C (Miyanishi and Cavers 1980).



Figure 1. *Portulaca oleracea*. Different growth stages on purslane wild growth.(Hortin & Hooper, 2011).

Purslane flower expansion is controlled by light intensity and temperature. The flowers can open between 9:00 am and 1:00 pm only on bright, hot days. Flowers are cleistogamous only under reduced light intensity. Purslane flowers are generally self-pollinated as no nectar is produced and the flowers are visited by only a few small insects. Even though purslane has no special structure for vegetative reproduction, it does have the ability to produce adventitious roots. These roots emerge only from the cut or broken surface of stem fragments and do not emerge from nodes or intact stems kept in continuous contact with moist soil (Miyanishi and Cavers 1980).

2.1.1 Purslane around the world

Purslane geographical distribution includes a wide range of both temperate and tropical regions (Holm et al. 1991). In Canada, purslane is found in every province, except Newfoundland, Fort Vermilion (Alberta) and Northern Territories, mostly due to the long cold periods and the short sun exposure. This plant is most commonly found in gardens, fertile fields and in row crops but is less common in waste places and along highways (Miyanishi and Cavers 1980).

Although purslane is considered a weed worldwide, its use as a leafy vegetable mostly occurs in Europe, Asia and Mexico. It is eaten both fresh and dry in salads, as cooked greens and in soups. The stems, leaves and flower buds are all edible. Purslane has a long history of use for human food, animal feed and medicinal proposes (Simopoulos 2004). Purslane cultivation and use are known around the world, mostly in Mediterranean countries. However, most people in modern societies limit their diet to a few cultivated vegetables. Still, production and use are limited in the United States and Canada (Fontana et al. 2006, Simopoulos 2004).

2.2 Purslane nutritional content

Although consumption of this plant is not widespread, the interest in its production has increased in recent years because of studies showing its high content of fatty acids, especially α-linolenic acid (ALA 18:3 *n*-3) and linoleic acid (LA 18:2 *n*-6) (Table 2). It has been demonstrate that purslane, wild growth, has the largest quantity of fatty acid in fresh leaves compared with common edible plants including borage (*Borago officinalis* L), primrose (*Oenothera paradoxa* Hudziok), sorrel (*Rumex acetosa* L.), beet greens (*Beta vulgaris* L.), spinach (*Spinacia oleracea* L.), buttercrunch lettuce (*Lactuca sativa* L.) and mustard greens (*Brassica juncea* L.) (Simopoulos et al. 1986, Simopoulos et al. 1995).

When comparing the nutritional content of purslane with other common vegetables, fruits or seeds including sunflower, avocado, olives or flax, the great nutritional value of purslane is evident (Table 1). In particular, it has a relatively high content of protein, carbohydrate and fiber. Furthermore, the fat content of purslane is

lower than all the plants compared, except lettuce; especially when compared with sunflower, flax and avocado (Table 1).

Gran	Protein	Fat	Fat Carbohydrate		Ash
Стор			(g)		
Purslane	26	4	50	11,5	20
Sunflower	24	47	19	3,8	4
Spinach	28	5,5	40	8	13
Avocado	2	12,7	8,5	6,7	1,6
Lettuce	2,1	0	3	0,5	1,2
Olive	1,6	22	19	0,1	0,1
Flax	19	35,5	35,4	6,8	3,5

Table 1: Nutrient contents estimated for 100g (FW) of plant tissue.

From Guerrero et al. 1998, Aletor and Adeogun 1995, Epstein 1971, Guil et al. 1998.

Purslane is also an excellent source of the antioxidants vitamin E, vitamin C, β carotene, and glutathione (Mohamed and Hussein 1994). One hundred grams of fresh purslane leaves contains 200- 460 mg of fatty acids and high amounts of α -tocopherol, absorbic acid, phenolic acids, flavonoids, β -carotene, betacianin, betaxantin, glutamine, methionine, folic acid, vitamins A and C (Cros et al. 2007;Agha et al 2010) (Tables 2 and 3). Purslane is also a rich source of the amino acids isoleucine, leucine, lysine, cystine, phenylalanine, tyrosine, threonine and valine.

Crop	Vit A	Vit B1	Vit B2	Vit C	α-Linolenic	Linoleic
Purslane ¹	15000	0,35	2,4	350	457	200
Sunflower ²	30	1,96	0,23	0	74	1200
Spinach ¹	50	0,7	2	600	138	26
Avocado ³	3	0	0	17	110	1440
Lettuce ¹	2200	0	0	15	0	0
Olive ³	0	0	0	0	64	810
Flax ²	0,03	0,17	0,16	0	280	55

Table 2: Content of vitamins and fatty acids of purslane, sunflower, spinach, avocado, lettuce, olive, flax (mg/ 100g FW).

Content of α -linolenic and linoleic acids¹ were measured in leaves, ² in seeds and ³ in fruit. From: Guerrero et al. 1998, Aletor and Adeogun 1995, Epstein 1971, Guil et al. 1996.

Table 3: Mineral content of purslane, sunflower, spinach, avocado, lettuce, olive and

Crop	Ca	Р	Fe	Mg	Na	К	Zi
Purslane	1500	550	29	0	55	1800	0
Sunflower	120	837	7,1	0	30	920	0
Spinach	800	415	80	0	650	4500	0
Avocado	12	52	0,5	29	7	485	0,6
Lettuce	26	30	0,7	10	3	208	0
Olive	1,3	0,1	0,6	0	38,9	1	0
Flax	220	415	23	0	0	0	0

flax (mg/100g FW).

From: Guerrero et al. 1998, Aletor and Adeogun 1995, Epstein 1971, Guil et al. 1998.

2.3 Purslane impact on health, pros and cons

The consumption of fatty acids and antioxidants such a flavonoids, betalains, carotenoids, vitamin C and polyphenols, all present in purslane, have high impact on health. These are said to prevent and treat several diseases (Galli et al. 1994), including coronary heart disease (De Lorgeril et al. 1994 2004), cardiovascular disease (Simopoulos 2008), hypertension (Appel et al.1993), renal disease (De Caterina et al. 1993), rheumatoid arthritis (Kremer 1996), chronic obstructive pulmonary disease (Shahar et al. 1994), and other inflammatory and autoimmune diseases (Dkhil et al 2011).

Although this edible plant presents great nutritional value, consumption of large quantities of purslane represents a considerable quantity of oxalic acid. The concentration of this acid has several negative effects in human health related with the occurrence of kidney stones, hyposidermia, and low plasma levels of iron and calcium (Palaniswamy et al. 2004). The extent to which foods high in oxalic acid are a potential health problem varies from person to person. Individuals with special vulnerability to oxalates, notably those with kidney disorders, gout, rheumatoid arthritis, or certain forms of chronic pain, need to be careful about their intake of oxalic acid. Normally healthy people need not to be concerned, unless, they are consuming unusually large amounts of oxalic acid on a long-term, continuing basis (USDA, 2011)

2.3.1 Fatty acids and their health impact in humans

The human consumption of polyunsaturated essential fatty acids (PUEFA) is an essential key for a balanced nutrition, enhancing growth as well as preventing several diseases that are life-threatening. These molecules have attracted considerable interest as pharmaceutical and nutraceutical compounds (Farmer 1994, Leon et al. 2001, Los and Murata 1998, Zheng et al. 2005).

Two of the most studied PUEFA are Omega-3 fatty acid (ω -3FA) and omega-6 fatty acid (ω -6FA). Both have been documented for their crucial role in physiology and central nervous system formation (Boden et al. 2005, Bourre and Dumont 2003) and also for preventing cardiovascular diseases (Galli and Marangoni 2006, Hu et al. 1999, Simon et al. 1995). More specifically, for ω -3FA acids, several studies have described their beneficial effect related to cancer, inflammatory bowel disease, rheumatoid arthritis, and psoriasis (Simopoulos 2002).

Omega-3 and omega-6 fatty acids have different properties; their main dissimilarity is based on the location of the first double bond, counting from the methyl end of the fatty acid molecule. For ω -3FA, the first double bond is between the 3rd and 4th carbon atoms and for the ω -6FA the first double bound is between the 6th and 7th carbon atoms (Simopoulos 2008).

The manifestation of ω -6FA in nature is mostly related to the seeds of plants, including coconut, cocoa and palm. In contrast, ω -3FA is mostly found in the

chloroplasts of green leafy vegetables and in some seeds including flax, chia, perilla and walnuts (Simopoulos 2008). Omega-3 is represented by Alfa linoleic acid (ALA) and omega-6 is represented by linoleic acid (LA); because of the specifically properties of each group of fatty acids, the ratio of them in human diet represent an important role (Simopoulos 1999). Numerous studies have pointed to the importance of a higher ALA/ LA ratio in the diet with a preferred intake of 1:1-2 ratios, compared to the usual ratio of 1:20 found typically in modern western diets (Liu et al. 2000, Simopoulos et al. 1992).

The current intake of ALA is much lower compared to ancient diets (Eaton and Konner 1985); the decrease of fish intake and the industrial production of animals fed with grains rich in LA, resulted in the commercialization of meat rich in LA and poor in ALA (Simopoulos 2002); and also, some current techniques in modern agriculture produce vegetables with high quantities of LA (Simopoulos 2004). These reasons may explain the poor ratio of ALA and LA consumed now (Anastacio and Carvalho 2013). Fortunately, some plants, like spinach and purslane, are known for having elevated quantities of ALA (Simopoulos et al. 1992).

2.3.1.1 Fatty acids in purslane

Purslane is a plant rich in PUEFAs, including ALA and LA, as well as antioxidants such as α -tocopherol, ascorbic acid, b-carotene and glutathione, plus minerals, vitamins and proteins (Palaniswamy et al. 2001, Simopoulos 2004). Other plants have been studied

for their ALA content, but purslane continues to be the terrestrial plant with the highest amount (Simopoulos et al. 1992, 2005, Ezekwe et al. 1999, Teixeira et al. 2010). Historically, purslane has been recognized for its importance in human diet, animal feed and medicinal purposes. The presence and concentration of ALA and LA in purslane varies depending on the accession, geographical distribution, developmental stage and environmental factors (Ezekwe et al. 1999, Liu et al. 2000, Palaniswamy et al. 2001)

2.3.1.2 Nitrogen effect on fatty acids concentration in plant tissue

The source of nitrogen provided to the plant is one factor affecting the quantity of PUEFA's in purslane. The type of nitrogen used and its ratio represent important ways for identifying new methods to produce purslane with desired characteristics. The source of nitrogen (NO₃- or NH₄+) in the growing medium can effect in the concentration of fatty acids in purslane (Fontana et al. 2006, Palaniswamy 2000, 2002, Szalai et al. 2010). Studies in algae (Piorreck et al.1984), spinach (Ahmed and Johnson 2000) and tomato (Clark 1936) also report the influence of the nitrogen form and concentration in the quantity of fatty acids in these plants.

2.3.1.3 Light effect on fatty acid concentration in plant tissue

Light is important in the regulation of fatty acids in plants. Different day lengths resulted in significant changes in the concentration of PUFAS in young pea leaves (Tremole et al. 1973). Central to this project, Fontana et al. (2006) determined the ALA concentration in purslane can be optimized when the day length varies between 14 and 16 hours.

2.3.2 Oxalic acid presence in plants tissue

Oxalic acid is an integral component of certain organisms. The occurrence of oxalate is common in plant tissue and several plants retain oxalate in their leaves, stems and roots (Libert and Franceschi 1987). Oxalate can be found in plant tissues as soluble oxalate, insoluble calcium oxalate or a mix of these two forms. Formation of these components depends mostly on the mineral element which the oxalate is bound to. Soluble salt are formed in the presence of potassium, sodium or magnesium; while calcium and iron produce insoluble salts (Moreau and Savage 2009). Oxalate may also be present as a free oxalic acid in plant tissues (Noonan and Savage 1999, Das et Savage 2013).

2.3.2.1 Oxalic acid and human health

The main concern about excessive presence of oxalate in plants, normally consumed in diet, is because they are known to be precursors of different diseases including calcium oxalate stones formation in the kidney, hyperoxaluria, arthritis, chronic deficiencies of calcium, magnesium and iron, skin irritation by direct contact, and in some extreme

cases, death may result if the amount of oxalate ingested is between 10 and 15 g (Libert and Franceschi 1987, Fassett 1973, Gontea 1968, Kumar et al. 2008, Noonan and Savage 1999).

2.3.2.2 Plants known for their content of oxalic acid

Plants in the Amaranthaceae, Araceae, Aizoaceae, Polygonaceae and Portulacaceae families are known to have high quantities of oxalic acid (Noonan and Savage 1999). The content of oxalic acid in plants used in human nutrition, such as taro (Colocasia esculenta) and sweet potato (Ipomoea batatas), have values of 278-575 mg/100 g and 470-781 mg/100 g, respectively (Mosha et al. 1995). Peanut greens, coriander leaf and sesame also have high values of oxalate in their tissues; 510 mg/100 g, 1268 mg/100g and 1750 mg/100g, respectively (Mosha et al. 1995, Gad et al. 1982, Meena et al. 1987, Ishii and Takiyama 1994). Other studies have reported the occurrence of oxalic acid in bamboo (163 mg/100g) (Judpresong et al. 2006), New Zeeland taro leaves (589 mg/100g) (Oscarsson and Sacage 2007) and oca tubers (162 mg/100g) (Savage et al. 2008). Studies made on purslane report oxalic acid content in the whole plant or the leaves, ranging from 255 mg/100 g to 1294 mg/100g (Bianco et al. 1998, Guil et al. 1997 1996, Ezekwe et al. 1999, Mohamed and Hussein 1992, Palaniswamy 2003, Poeydomenge and Savage 2007).

The wide range of values for oxalate content in plants has been attributed to accession variation and environmental conditions. For example, two accessions of spinach differed considerably, one accession, 'Winter gianta', contained 400 to 600 mg/100g, while the other accession's level ranged from 700 to 900 mg/100 g (Watanabe et al. 1994). Differences in oxalic acid levels have been related to the season which the plant is grown, as expressed in the recorded difference between summer and fall accessions (Aletor and Adeogun 1995). Different theories have been developed regarding to the formation of excessive oxalate in plants, but most are related to excessive calcium uptake (Olsen 1939) and the source of nitrogen, including the ammonium to nitrogen ratio and the total nitrogen (Kick and Massen 1973, Clark 1936, Gilbert et al. 1951).

2.3.2.3 Nitrogen effect on oxalic acid concentration in plants

The existence of a strong relationship between the quantity of oxalic acids and nitrate as a nitrogen source in the soil was reported as early as 1943 (Pierce and Appleman 1943). More recent studies with spinach showed that the nitrate to ammonium ratio in the plant mineral nutrition can affect oxalate levels in leaf and stem tissues (Ahmed and Johnson 2000). Similarly, purslane also accumulates water-soluble oxalic acids in considerable quantities in leaves and shoots, but when purslane is grown with different forms of nitrogen, the levels of oxalate in leaves and shoots are reduced (Fontana et al. 2006, Palaniswamy et al. 2004).

2.4 Purslane growth in controlled environment

Plant growth is influenced principally by the presence of water, carbon dioxide, oxygen, sunlight and mineral elements from the soil; the manipulation of these factors will empower the plant to increase or decrease its growth (Singh 2005, Taiz and Zeiger 2002). Two of these factors will be examined in the growth of purslane: nitrogen source and photoperiod.

2.4.1 Nitrogen in the growth of purslane

Nitrogen most often limits the growth and development of plants because nitrogen is an essential component in numerous organic compounds like proteins, nucleic acids, chlorophyll, and growth regulators (Rajan 2000). Nitrogen physiological efficacy depends on several factors: (1) efficiency of biomass formation, (2) carbohydrate partitioning, (3) nitrate-reduction efficiency, (4) remobilization of protein nitrogen from senescent tissues, (5) transport and storage functions, and (6) absorption forms on the substrate (Novoa and Loomis 1981).

Nitrogen can be absorbed by plants as both ammonium (NH₄⁺) and nitrate (N0₃⁻). The absorption efficiency of NH₄⁺ and N0₃⁻ is strongly related with the mobility of each form, NH₄⁺ gets bound to negatively charged soil particles thus makes it relatively immobile; on the other hand, N0₃ is mostly repelled by the soil particles, aiding its mobility to reach the plant roots. Other internal and external factors known to affect uptake of NH₄⁺ and N0₃⁻, are nitrogen and carbohydrate status in the plant, temperature, O2 level and rhizosphere pH (Arnon and Stout 1939, Taiz and Zerger 2002).

The effects of nitrogen on plant production are derived from biochemical, physiological, and morphological processes. At the biochemical level, the high cost of nitrogen assimilation and the difference in cost of nitrate reduction between roots and leaves appear as important issues for nitrogen use efficiency (Novoa and Loomis, 1981).

Different ratios of the N0₃-/NH₄+ play an important role in the growth of purslane (Szalai et al. 2010). Purslane growth varied among different ratios of N0₃-/NH₄+ (100:0, 75:25, 50:50 and 25:75),with the greatest growth occurring when grown under a ratio of 100:0; but other characteristics, important for this project, such as the fatty acid and the oxalic acids concentrations, presented favorable values when purslane was grown at 50:50 or 25:75 N0₃-/NH₄+ (Fontana et al. 2006, Palaniswamy 2004, Szalai et al. 2010). As a result, these two ratios were studied and described in this work, as expressed in the growth, fatty acid content and oxalic acid content in purslane.

2.4.2 Light effect in the growth of purslane

Light or photoperiodism is a physiological reaction managed by several photoreceptor proteins (phytochrome or cryptochrome), that allow plants to recognize changes in light length or photoperiod (Bewley 1985). Many functions in plant growth are affected by photoperiodism; one of the most relevant is the biosynthesis of the phytohormone gibberellin. This is known to regulate several aspects in plant development including dormancy, germination, flowering, and stem elongation. It has been shown that photoperiod can affect gene transcription for specific steps in the biosynthesis of gibberellin (Hopkins and Huner 2004, Taiz and Zeiger 2002).

The influence of photoperiod on carbon quantity and plant growth has been investigated in several species, revealing that photoperiod affects the growth of wheat, tomato and lettuce with long photoperiods increasing plant growth (Bugbee 1992, Bubgee and Salisbury 1988, Knight and Mitchell 1988). Shorter periods of light have been favorable for the growth of soybean, potato and rice. Also, some plants develop intolerance to very long photoperiods, including some accessions of potato (Chatterton and Silvius 1979 1980, Grange 1985, Rufty et al. 1984, Logendra et al. 1990). Other plants, like bean, are sensitive to photoperiod, expressing higher growth in day-neutral photoperiods (Pessarakli 1999 2002, White and Laing 1989). This response is truly photoperiodic rather than being a function of total light duration. When plants are grown under short days and long nights, but interrupted by a brief period of light, the growth of the plants is not disturbed (Wheeler et al. 1986, Hicklenton and Wolynetz 1987, Hendricks and Taylorson 1972).

Plants have an ability to adjust partitioning carbon when exposed to different photoperiods as found with tomatoes when exposed to photoperiods of 8, 16 or 20 hours each day (Logendra et al. 1990). Those exposed to the shortest light periods retained higher proportion of their photosynthates for later export during the long dark period, thereby maintaining some supply for growth and maintenance during the dark period. When grown under low light intensity for 14-16 hours, purslane growth increases as well as other nutritional components (Palaniswamy et al 1998, Palaniswamy et al 2000, Palaniswamy et al 2004).

2.4.3 Calcium oxalate crystals generated as bio-minerals

Calcium (Ca) oxalate is an ionic crystal, derivate from oxalic acid and almost insoluble in water. It has been observed in multiple members of the five kingdoms in nature and it is considered the most frequently occurring bio-mineral in plants (Franceschi and Horner 1980). At least 215 plant families have some species containing Ca oxalate crystals in different plant tissues (McNair 1932). Crystals are found in the vacuoles of certain specialized cells called idioblast (Foster 1956) The formation of Ca oxalate crystals has been found to be an intracellular process associated with some type of membrane system; a membranous complex is formed in the central vacuole which later gives rise to membrane chambers in which the crystal develop (Chiu and Falk 1975, Eilert 1974). Also, the presence of tubules, with modified plastids and enlarged nuclei, has been reported as an important component in the formation of Ca oxalate crystals (Franceschi and Horner 1980). Crystals are widely distributed in plant tissues and can be found in woody tissues of tropical woods (Chattaway 1956), bark and secondary xylem of *Acacia Zenegal* (Wattendorff, 1978), fruits and floral buds (Stebbins et al. 1972), floral organs including anthers and ovaries (Calazewska 1934), leaves and roots (Arnott 1976, Honner and Franceschi 1978), and bark, petioles, callus tissue, old roots, fruits and pedicels of apple (Stebbins et al. 1972).

Two different hydration states have been attributed to Ca oxalate, monohydrate (whewellite) or dehydrate (weddellite) (Frey-Wyssling 1981, Arnott 1982). Ca oxalate crystals shape are usually found as raphide, styloid, prisms, crystal sand and druses but other shapes do appear as variation of these forms (Franceschi and Harry 1980, Haberlandt 1914). The spatial distributions, as well as the morphology of the crystals in the plant are specific within taxon and they are often used in plant classification, as they could be specific for a given species (Heintzelman and Howard 1948).

Certain succulent species can accumulate enormous quantities of inorganic material, as much as 85% of its dry weight as Ca oxalate (Cheavin 1938). Purslane is a succulent plant that belongs to the Portulacaceae family. It is an annual, prostrate herb,

with thick tap root; reddish succulent stems and toothless succulent leaves (Miyanishi and Cavers 1980).

Purslane has been described as an edible plant with a great nutritional value in alimentation (Simopoulos 2004, Mohamed and Hussein 1994), which also has an elevated accumulation of oxalic acid, involved in the formation of Ca oxalates crystals (Palaniswamy et al. 2004, Franceschi and Harry 1980).

Little is known about the functions of the Ca oxalates in purslane, but generally crystals are proposed to have an effect in calcium regulation, ion balance (sodium and potassium), plant protection, tissue support (plant rigidity), detoxification (heavy metals), and light gathering and reflection (Franceschi and Harry 1980, Francescho 1989, llarslan et al. 1997, Volk et al. 2002, Finley 1999, Nakata 2003).

Although purslane presents great nutritional value, consumption of large quantities represents a considerable amount of oxalic acid. The concentration of this acid has several negative effects in humans' health, when oxalate is ingested from vegetables; it cannot be metabolized and is excreted by the kidney into urine, where it binds to calcium forming an insoluble salt that may precipitate to form kidney stones (Massey et al. 2001). Other health issues have also been attributed to oxalic acid intake including the development of hyposidermia, hypocalcemia, and low plasma levels of iron and calcium (Palaniswamy et al. 2004, Nutridata Laboratory 1984).

3.0 MATERIALS AND METHODS

3.1 Seed material

Different accessions of purslane were acquired thanks to collaboration of researchers from Iran, Syrian, Afghanistan, Saudi Arabia and local markets in Quebec (Table 4). Seeds were accessed from the Israeli Gene Bank (Israel) and the Seed Bank UPCT (Universidad Politecnica de Cartagena, Spain). The local accessions from Canada were collected in three different locations in Quebec during the 2012 summer.

3.1.1 Seed stocks

Seed stocks of the different accessions were increased by sowing a small set of seeds of each accession and bulking the seeds produced. Seventy-two celled seedling trays (1.5"Lx1.5"Wx2.25"D) were filled with a mixture of 80% agro mix (Agro Mix® Potting and Seeding Mix, Fafard) and 20% Perlite. Three seeds were sown per cell and replicated four times. After 60 days of growth, seeds were collected and stored at -4°C. Approximately, 200 seeds were obtained per accession. Prior use, seeds were treated with a 2% sodium hypochlorite solution and washed three times with distilled water.

3.2 Plant growth and harvest

Three replicas of 20 seeds of each accession were sown into 4" Kord traditional standard pots (4" x 3-1/2") containing the above soil mixture. Seeded pots were placed

into growth chambers at 24°C/20°C day/night and 50% relative humidity, one chamber with 12 hours photoperiod and the second chamber with 16 hours photoperiod. The light intensity in both growth chambers was 1100 µmoles/m^{2/s} provided by incandescent and fluorescent bulbs.

Plants were watered daily with a nutrient solution containing CaCl₂, KCl, MgCl₂, KH₂PO₄. After 12 days, two different ratios of nitrate and ammonium were added to the initial nutrient solution and the nitrogen treatments continued for 34 days (Table 5). Thirty-five ml /pot of the nutrient solution were applied twice (morning and night) every three days. During the last five days of growth, the plants were watered with tap water to remove remaining mineral particles over the plant.

After 51 days of growth, the plants were harvested. This growth period was determined to correspond to the purslane's peak greatest growth, as well as the optimum for fatty acids and oxalic acids determination (Szalai et al. 2010). The standardized number of harvested plants was three, corresponding to the minimum number of desired-growth plants in all the pots. Therefore, three plants were harvested in each of the three replicate pots to provide a total of nine plant samples. The experiment was repeated a second time.

3.3 Parameters measured

3.3.1 Plant growth

Immediately after harvest plant fresh leaf weight (FW g), fresh stem weight (FW g), fresh root weight (FW g), stem height (cm) and root length (cm), total fresh weight (FW g) and total dry weight (DW g) were measured. Chlorophyll content was also measured with a SPAD 502 Plus SPAD 510 chlorophyll meter (Minolta, Tokyo, Japan).

3.3.2 Fatty acid procedure

3.3.2.1 Fatty acid extraction for GC Analyses

The samples were freeze dried and stored at -80°C until use. The material was grounded to a fine powder with a mortar and 200 mg of sample were added into 15 ml plastic tubes with a tight sealing cap, then 5 mL of hexane were added. Tube contents were mixed and shaken at 50 rpm at room temperature for 30 min. The mixture was centrifuged at 3500 rpm for 15 min and filtered using Whatman 1 filter paper with1 g of anhydrous sodium sulphate. The filtrate or hexane portion was transferred to 15 mL plastic tubes and 1,5 mL of sodium methoxide reagent (26 g/L methanol) was added. The tubes were cap tightly and vortex for 2 min to methylate the fatty acids, with 10 s intervals for better separation. Five mL of NaCl saturated solution was added to the tube and shaken vigorously for 15 s. The tubes were let to stand for 10 min. The sample was then filtered through a 0.2 µm filter to remove impurities and the resulting hexane

layer was transferred to vials for subsequent gas chromatography analysis. The vials were frozen at -80°C until analyzed.

3.3.2.2 Fatty acid analyses

A GC Hewlett Packard HP 6890 series (Agilent, CA) was used for analyzing the fatty acid profiles of the samples, using Varian CP-Sil 8C8 WCOT fused silica capillary column (30m x 250µm x 0.25µm ID). The gas carrier was helium and the injection volume was 1.0 µl. The initial temperature was 60°C which was raised at10°C /min to 150°C and held for 10min, after the temperature was raised to 230°C at 3°C /min and held for 20 min. The peaks on the samples chromatograms were compared with the retention times of standard fatty acid mixture (Mortley et al. 2012). Fatty acids were expressed as relative percentages. A total of 216 samples were analyzed.

3.3.3 Oxalic acid procedure

3.3.3.1 Sample preparation

Plant samples were prepared following the method of Ilarslan et al. (1997). Dried ground plant material (0.1-0.2 g) was mixed with 5 ml of de-ionized water and the mixture was vortexed. After, 5 ml of EDTA (10 mmol/L) were added to the mixture. The pH was checked and kept between 5 and 7. Five ml of each diluted sample were pipetted to 15 ml plastic tubes and mixed for approximately five minutes and incubated at room temperature for 24 hours. The tubes were then centrifuged at 3000 rpm for 10 min. The supernatant was used for oxalic acid determination.

3.3.3.2 Oxalic acid determined

An oxalate kit purchased from Trinity Biotech (Oxalate Urinalysis Diagnostic kit: Procedure 591, Trinity Biotech, USA) was used for determining the concentration of oxalic acids in leaves and stems of purslane. Two reagents were acquired with the kit: Reagent A (DMAB 3.2 mmol/L (3 –dimethylamino benzoic acid) + MBTH 0.22 mmol/L (3-methyl-2-benxothiasolinone hydrozone), pH 3.1±1) and Reagent B (Oxalate oxidase (barley) 3000 μ /L + peroxidase (horseradish) 100.000 μ /L); these two reagents were prepared and storage following instructions from the kit, before used they were warmed and kept in 37°C. Two ml test tubes were labeled for blank, standard and sample; after 50 μ L of Reagent A was added to each tube, following of 50 μ L of de-ionized water in the blank tube, 50 μ L of standard (0.5 mmol/L) in the standard tube and 50 μ L of supernatant in the sample tube. The blank and standard tube were measured once each 100 samples. A total of 216 sample tubes were prepared.

Subsequently, 0.1 ml of Reagent B was added to all the tubes and immediately mixed by gently inversion. All the tubes were incubated at 35°C for 5 minutes. A dilution of 10 volumes was necessary; 0.1 ml of mix was transferred to cuvettes and after 0.9 ml of de-ionized water was added. The absorbance of blank, standard and sample were
determined at 590 nm in an ultraspec 2100 pro uv/visible spectrophotometer (McGill University, Canada). Measurements were made twice to obtain consistence absorbance. The absorbance of the samples and standards were corrected by subtracting the blank absorbance. The concentration of oxalic acid was determined in milligrams, as shown in kit 591, per 100 g fresh weight (FW).

3.3.4 Oxalic acid crystals identification

3.3.4.1 Plant Material

Specimens of purslane from local weedy populations were collected in Sainte-Anne-de-Bellevue, Quebec in April 2012. The plants were propagated vegetative and after three months, the many-seeded capsules were harvested as described above in *3.1.1*. In June 2012, seeds were sown into agro mix soilless mixture in a seedling tray as describe above in *3.2*. After four months of growth, proximal and basal leaves from four plants were removed and washed several times in distilled water prior to clearing and staining.

3.3.4.2 Leaf clearing and staining

Both young and old leaves were cut into 1cm² pieces containing a portion of the mid vein. Cut pieces were placed into 60 ml glass specimen bottles and 40 ml of 80% ethanol was added as described by Tidsall and Donnelly (1988). The material was

autoclaved for 10 min at 130°C, and when removed from the autoclave, approximately 10 ml of ethanol remained in the bottles. The ethanol-chlorophyll solution was filtered and the vegetal material was removed, rinsed four to five times in distilled water.

For dissolution of protoplasm, cleaned leaf sections were submerged in a 2.5% NaOH solution for 20 min at 50°C. The NaOH solution was filtered, using a small strainer to prevent damage to the leaf pieces; and the material was rinsed with distilled water as described above. After 10 min., the pieces were submerged in 40 ml 2.5 % NaClO solution at room temperature for 20 min. The remaining NaClO was removed and the tissues were washed five times. After washing, the tissues were stained with aqueous safranin (10 mg/L⁻¹), rinsed again several times, soaked in distilled water and then semi-permanently mounted in glycerol (Tidsall and Donnelly 1988).

3.3.4.3 Light Microscopy

Druses where visualized using an Olympus BH-2 polarizing microscope. Samples were mounted on glass slides and inspected using 10x, 20x and 40x dry objectives. Photographs were taken using a Canon EOS Rebel Toi color camera.

3.4 Statistical treatments

Data were combined and analyzed using a factorial model (SAS Institute, 2013) with significance determined at the 0.05 level of probability, the packed procedure Proc

mixed was used. The entire experiment was repeated once and there were no differences in the treatment effects between experiments, and therefore results from the two experiments were combined [Levene's test ($P \ge 0.05$)]. The statistical significant difference between means was determined according to Bonferroni's and Scheffe's multiple range test ($P \ge 0.05$).

4.0 RESULTS

4.1. Purslane growth

4.1.1 N0₃/NH₄⁺ ratios, Light and accessions effect on purslane growth

The study of different growth characteristics in purslane allowed us to determine how the main effect of NO_3 -/NH₄+ ratios (50:50 and 25:75), the main effect of photoperiod (12 and 16 hours light) and the main effect accession (nine different accessions) on the growth of purslane, as single factors or their interactions.

The growth of purslane was not affected by interactions amongst NO_3 -/NH₄+ ratios (50:50 and 25:75), photoperiod (12 and 16 hours light) and accessions. Similar results were found when the exposure time and the accessions were combined. For the other interaction and effects, a significant statistical difference was found.

4.1.2 N0₃/NH₄⁺ ratios and photoperiod interaction

When the N0₃-/NH₄+ ratios and photoperiod were studied for their interaction and how this affected the growth of purslane, certain combinations were significantly different (Table 6). Plants grown under a N0₃-/NH₄+ ratio of 50:50 and 16 hours light had the highest values for stem height, chlorophyll content, leaf FW, stem FW, total FW and total DW. These plants were statistically different from the other three combinations of growth conditions. When plants were exposed to longer light periods, plant growth, in general, increased.

4.1.3 NO₃/NH₄+ ratios and accession

Accession 1 (Local accession # 1) grown under a 50:50 N0₃-/NH₄+ ratio had the highest values for stem height, leaf FW, stem FW, total FW and represented the only accession with statistical significant difference among the nine accessions within the two N0₃-/NH₄+ ratios [($P \ge 0.05$) according to Bonferroni's multiple range test)] (Table 7).

4.1.4 NO₃/NH₄+ ratios effect

Different N0₃-/NH₄+ ratios influenced the growth of purslane (Table 8). Stem height (SH), leaf fresh weight (LFW), stem fresh weight (SFW) and root fresh weight (RFW) were affected. Plants grown under 50:50 N0₃-/NH₄+ ratio had higher values in chlorophyll content, stem height, root length, leaf FW, stem FW and root FW. Even so, there was no significant difference between the two ratios of N0₃-/NH₄+ in chlorophyll content and root length.

4.1.5 Light Exposure Effect

Plants in this study, grown under 16 hours light exposure, had higher chlorophyll content, and greater stem height, leaf FW, stem FW, and root FW values (Table 9). Root length was the only measured variable that was higher when exposed to 12 hours light, but root length was not significantly different between the two light exposure hours.

4.1.6 Accession effect

The use of different accessions exhibited a clear dissimilarity in the general growth of purslane (Table 10). Accessions 1 and 9 had the highest total DW, 0.14 and 0.15 g, respectively, significantly different from Accessions 5 and 7 (0.08 and 0.09 g). Accession 1 had the highest total FW value (6.9 g) and was significant different from Accessions 2, 3, 5 and 8 (4.11, 3.39, 4.97 and 5.08 g, respectively). The chlorophyll content in Accession 3 (27.13) was significantly different from all the other accessions

Differences in root length and stem height were observed among accessions (Table 11). Accession 7, with the highest value (5.37 cm), had significant longer roots than Accessions 9 and 4 (3.79 and 3.90 cm). Stem height was greatest in Accession 1 (18.23 cm) and significantly higher than Accessions 3, 4 and 5 (9.79, 12.09 and 9.40 cm, respectively).

Fresh root weights in purslane were between 0.14 g and 0.31 g (Table 12). Accession 7 had the highest root weight and was statistically higher than Accessions 2, 3 and 5. Accession 1 had the highest stem fresh weight that was significantly greater than stem weight of Accessions 2, 3, 4, 5, and 8 (Table 12). Accession 1 also had the highest leaf fresh weight, statistically greater than leaf fresh weight of Accessions 2, 3 and 5 (Table No 12).

In summary, Accession 1 or Local accession #1 was the best in terms of crop growth when compared with the other eight accessions. Accession 1 had the highest

values of total FW, total DW, stem FW and leaf FW, making it the accession that responded the best to conditions of nitrogen/ammonium and light exposure used in this work. Accession 3 or Var. 'Golden', showed the lowest values in chlorophyll content, total FW, total DW, root FW, stem FW, leaf FW and root length.

4.2 Fatty acid analysis

The leaf and stem fatty acid compositions in purslane were affected by N03-/NH4+ ratios in the nutritive medium, as well as the hours of light exposure and the different accessions (Table 4). Each main effect was study as individually as well as an interaction. The study of the interaction among the three main effects, N03-/NH4+, light exposure and accessions, displayed significant differences for some of the fatty acids.

4.2.1 Effect of the interaction between N0₃:NH₄+ ratios, light exposure and accession on purslane fatty acid content

The analysis of the interaction between the three main factors N03-/NH4+, light exposure and accessions exposed a significant difference in the content of linoleic acid (18:2) and α -linolenic acid (18:3) known also as LA and ALA, respectively. The highest quantity of LA was found in the Accession 3 or Var. 'Golden' (33%) when grown under a N03-/NH4+ ratio of 50:50 and 12 hours light exposure (Figure 2). Accession 1 or Local accession #1 had the second highest level of LA (30%) when grown under the same

condition. These two accessions had values significantly higher quantities of LA when compared with all the other accessions grown under the two conditions of light exposure and the two ratios of N03-/NH4+, except Accession 1 grown under a N03-/NH4+ ratio of 25:75 and 12 hours light and 16 hours light with 25.5 and 23.7 % of LA; and Accession 5 grown under a N03-/NH4+ of 25:75 and 16 hours light with 23.5% of LA (Figure 2).

All accessions grown under a N03-/NH4+ ratio of 25:75 and 16 hours light had levels of ALA between 26% and 41% (Figure 3). Accession 6 or 'Local accession #2' had the highest value of ALA and was followed by Accession 1, 5 and 9 with 36 % each.

Plants grown under a N03-/NH4+ ratio of 50:50 and 16 hours light had between16% and 34% ALA, Accession 2 (Var. 'Tall Green') and 8 (Afghanistan accession), each with 34% ALA were significantly higher than all plants grown under 12 hours light, across the two N03-/NH4+ ratios (Figure 3).

When the quantity of LA and ALA were analyzed for their ratio, Local accession # 1 had a ratio of 1:0.8, with 25.48% of LA and 21.19% of ALA, which represented the closest ratio to 1:1 LA:ALA. When analyzing plants that had high ALA, , all plants grown under a N03-/NH4+ ratio of 25:75 and 16 hours light, the LA:ALA ratio range from 0.3:1 to 0.65:1 for (Figure 4), Accession No 1 or Local accession #1 had the most suitable proportion (0.65:1 LA:ALA), this means that for each 100 g serving of purslane, from the total of the fatty acids present in the plant, the quantity of ALA or α -linolenic acid will be

35% times greater than the proportion of LA or linoleic acid, which will represent a higher amount of ω -3FA compared to the amount of ω -6FA (Figure 4).

4.2.2 Effect of NO₃:NH₄⁺ and light exposure interaction on the concentration of fatty acids in purslane

The contents of palmitic (C16:0), linoleic (C18:2) and α -linolenic (C18:3) acids were affected by the interaction amongst N03⁻/NH4⁺ and light exposure (Table 13). The content of these three fatty acids was always significantly higher in the plants grown under 16 hours light and a ratio of 25:75 N03⁻/NH4⁺. Plants grown under these conditions had 30% more palmitic acid than plants grown under a N03⁻/NH4⁺ 50:50 ratio and 12 hours light and a N03⁻/NH4⁺ ratio of 25:75 and 12 hours light. Plants grown under N03⁻/NH4⁺ ratio of 25:75 and 16 hours light offered 40% more LA than plants grown under the same ratio of N03⁻/NH4⁺ but 12 hours light, and 21% and 10% more than plants grown under a N03⁻/NH4⁺ of 50:50 combined with 16 and 12 hours light, respectively. The content of α -linolenic acid was 52% higher in plants grown under 16 hours light with a ratio of N03⁻/NH4⁺ 25:75, compared with plants grown under the same N03⁻/NH4⁺ ratio but 12 hours light. With the 50:50 N03⁻/NH4⁺ mixture, there was 29% less linolenic acid at 12 hours light and 10% less at 16 hours (Table No 13).

4.2.3 NO₃:NH₄+ and light exposure factors and their effect on the concentration of fatty acids in purslane leaves and stems

The N03-/NH4⁺ ratio significantly affected the quantities of palmitic acid (C16:0), linoleic acid (C18:2) and α -linolenic acid (C18:3) fatty acids in purslane .The concentration of C18:3 (or ALA) was significantly higher in plants grown under a ratio of 50:50, while the concentration of C18:2 (or LA) was similar with the two ratios, while fatty acid C16:0 values were significantly higher in plants grown under a ratio of 25:75. The other four fatty acids, myristic, palmitoleic, stearic and oleic were not affected by the N03-/NH4⁺ ratio (Table 14).

The hours of light exposure also affected fatty acid concentration of leaves and stems of purslane. As with the ratios of N03⁻/NH4⁺, the fatty acids affected by photoperiod were C16:0, C18:2 (LA) and C18:3 (ALA). The content of fatty acids was always higher at 16 hours light exposure. C16:0 concentration was significantly greater with 16% more content the content of C18:2 was significantly higher by 15% and the content of C18:3 was 37% higher when compared with the plants grown under 12 hours of light. Other fatty acids were not affected by photoperiod (Table 15).

4.2.4 Effect of accession in the concentration of fatty acids in purslane leaves and stems

Total fatty acids (percentage %) means across all the nitrogen forms and light exposures for each accession are shown in Figure 5. The contents of palmitoleic (C16:1), stearic (C18:0) and oleic (C18:1) ranged between 0.09%-1.4% for C16:1; 1.12%-2.7% for C18:0 and 3.02%-5.2%- for C18:1. Concentrations of myristic (C14:0), palmitic (C16:0) and linoleic (C18:2) ranged between 5.4%-11.5% for C14:0, 8.6%-12.3% for C16:0 and 9.9%- 23% for C18:2. The content of linolenic acid (C18:3) was approximately 45% higher than the quantity of its predecessor fatty acid, linoleic, that ranged between 21.5% and 30.9%.

4.3 Oxalic acid analysis

4.3.1 N0₃:NH₄⁺ ratio, light exposure and accession effect on purslane oxalic acid content The effect of two ratios of N0₃:NH₄⁺ (50:50 and 25:75) jointly with two photoperiods (12 and 16 hours light) and 9 different purslane accessions on the oxalic acid content in the plant tissue was determined in this study. It was possible to identify a statistical significant interaction between the three main factors: N0₃:NH₄⁺ ratios, photoperiods and accessions. The effect of nitrogen on the quantity of oxalic acid in purslane depended on the effect of photoperiod and accession (Figure 6). Accession 3, had the lowest oxalic acid concentration (95 mg/100g FW oxalic acid) when Accession 3 was grown under a N0₃:NH₄+ ratio of 50:50 and 12 hours light. This same accession grown under 16 hours light exposure and a N0₃:NH₄+ of 25:75 produced 86% more oxalic acid. When this accession was grown under 12 hours light and the N0₃:NH₄+ ratio was 25:75 and 16 hours light and the N0₃:NH₄+ ratio was 50:50 the concentration of oxalic acid had values of 447.5 and 371.37 mg/100g FW (Figure 16).

Accessions 1 and 2 had the highest values of oxalic acid (813.9 and 807.37 mg/100g FW) when grown under a N0₃:NH₄+ ratio of 50:50 and 16 hours light. These plants had 88% more oxalic acid that Accession 3 with 95 mg/100 g FW.

4.3.2 Effect of N0₃:NH₄+ and light exposure interaction on the concentration of oxalic acid in purslane

When the interaction between the main effect nitrogen and light exposure was studied, the quantity of oxalic acid in the leaves and stem tissues of purslane was 40% less in plants grown under 12 hours light and 50:50 NO_3^{-}/NH_4^{+} ratio, than plants grown under 16 hours light and 25:75 NO_3^{-}/NH_4^{+} ratio (Table 16). The plants grown under 12 hours light and NO_3^{-}/NH_4^{+} of 50:50 and 25:75 had significantly less oxalic acid content. Plants grown under NO_3^{-}/NH_4^{+} ratio of 50:50 had 20.6% less oxalic acid in their tissue than plants grown under NO_3^{-}/NH_4^{+} ratio of 25:75 (Table 16). There was no interaction effect

with N0₃:NH₄+ 50:50 and 25:75 ratios on oxalic acid content when plants were grown under 16 hours light.

4.3.3 NO₃:NH₄⁺ and light exposure factors and their effect on the concentration of oxalic acid in purslane

When the main effect N0₃:NH₄⁺ was studied, the quantity of oxalic acid was lowest (488.36 g/100 mg FW) for plants grown under 50:50 N03:NH4+ ratio. This value was statistically lower than the quantity of oxalic acid in plants grown under the N0₃:NH₄⁺ ratio of 25:75 (Figure 17).

Light exposure affected the growth of the plants too. Plants grown under 12 hours light had 31% less oxalic acid in their leaves and stem tissues than plants grown under 16 hours light, representing a statistically significant difference (Table 17).

4.3.4 Effect of accession on the concentration of oxalic acid in purslane

There were statistically significant differences in oxalic content among the nine accessions of purslane studied (Table 4). When the accessions were analyzed as a single factor, the quantity of oxalic acid in tissues of Accession 3 was 412.07 mg/100g FW; this value is 37% smaller than the quantity of oxalic acid found in Accession 2 with 659.044 mg/100g FW of oxalic acid, the highest value found. Accession 6 had the

second lowest value, with 430.94 mg/100g FW, 34% less oxalic acid than Accession 2 (Table 18).

4.4 Oxalic acid crystals identification

Oxalate crystals were found in old and young leaves from the wild specimens of purslane. The druses-shaped crystals, more or less round, observed on purslane leaves were located mostly under and above the main vain of the leaf and in less quantity, in the spongy mesophyll. The general aspects and the locations of these druses in the leaves are shown in Figures 7 through 10.

Hundreds of microcrystals, tightly packed together, form the microstructure found in the tissue, known as druses. Different sizes of these rounded shapes were distinguished; the general aspect of a single druse varies among species, especially with respect to size and angles between individual crystal faces, as can be seen in Figures 10 and 11. The shape of the druse can help to differentiate the hydration state of the crystal, between the monohydrate state (whewellite) and the dehydrate state (weddellite). Druses present in purslane have stellate shapes with individual crystallites having acute sharp points emerging from the center of the druse (Figure 11).

5.0 DISCUSSION

5.1 Purslane growth under a controlled environment

The results of this study showed that nine accessions of purslane grown under two different fertilizer treatments and under two different photoperiods don't have a statistically significant dependence between them; this is, the main effect accession doesn't depend on the nitrogen source-concentration and the hours of light; the main effect nitrogen source-concentration doesn't depend on the nine accessions and the hours of light; and the main effect photoperiod doesn't depend on the nine accessions and the nitrogen source-concentration.

The main effects nitrogen source-concentration and photoperiod, both with two levels, had a statistically significant interaction. The plants grown under 50:50 N0₃- /NH₄+ ratio and 16 hours light exposure showed the greatest values of total FW, total DW, leaf FW, stem height and chlorophyll content.

Different sources of nitrogen in the nutrient solutions cause physiological and morphological changes in the plant, which may affect the possible optimization of gas exchange rate, impacting the plant's growth (Radin and Boyer 1982). When plants are under nitrogen stress, the photosynthesis activity gets reduced in leafs, as well as the cellular expansion, the presence of water in leafs, and may also cause secondary deficiencies of other nutrient essential for the plant growth (Radin and Parker 1979,

Radin and Boyer 1982); this stress may explain why plants grown under N0₃-/NH₄+ ratio 25:75 had lower values in the general growth (Palaniswamy et al 2000, 2004).

Previous studies of tomato (Claussen 2002) indicated that the ammonium form of nitrogen caused a decrease in total dry weight. The result of this study agrees with studies made on purslane and spinach (Ahmed & Johnson 2000, Palaniswamy 2004). Plants had a better growth when ammonium is used as 50:50 ratio of NO₃⁻ and NH₄⁺. Nitrogen in ammonium form can be used by plant in the synthesis of amino acids and amines, while the nitrate form needs to be reduced using, approximately, 25% of either photosynthetic or mitochondrial electron transport capacity (Bloom 1994).

When nitrogen is supplied in a balanced mixture of cations and anions, a reduction in the rapid raise of the pH of the medium is controlled; this raise is commonly observed when the nitrogen is supplied only as nitrate anion (Epstein 1999, Raven and Smith 1976). Even when the pH of the medium is kept neutral, most plants grow better if they have access to both N0₃-/NH₄+ because absorption and assimilation of the two nitrogen forms promotes cation-anion balance within the plant (Asher and Edwards 1983, Raven and Smith 1976). Even though, the maximal uptake for ammonium occurs at neutral pH values and its uptake is depressed as the pH falls, this, when NH₄+ is the only source of nitrogen, the growth of the plant will be repressed in acidic soils (Bloom 1994, Raven and Smith 1976, Weir et al. 1972).

As seen in purslane, a combination of $NO_{3^{-}}$ and $NH_{4^{+}}$ in an appropriate ratio, in this case 50:50, appears to benefit plant growth, as reported by several research groups (Gashaw and Mugwira 1981, Ikeda and Osawa 1983, Palaniswamy 2004, Salsac et al. 1987).

Plant growth is also affected by photoperiod; even though, studies related to photoperiod are mostly interested in the inhibition or activation of flowering, few works describe how photoperiod affects the plant's growth. The influence of photoperiod in this work had similar results to those reported by Palaniswamy et al. 1998, 2000 and 2004. The growth of purslane was higher when the plant was grown under 16 hours light and 8 hours darkness.

An increase in total FW in plants exposed to16 hours light was also present in tomato, having almost twice the weight of plant grown under shorter light periods (Hurd1973). The same response has been found in several grass species including perennial rye grass, *Lolium perenne* (Ryle 1966), timothy, *Phleum pratense*, and Kentucky bluegrass, *Poa pratensis* (Hay and Heide 1983, Heide et al. 1985).

A study made by Langton et al. (2003), measured directly *in situ* the leaf greenness of geranium (*Pelargonium*), using a portable chlorophyll meter (Minolta SPAD-502), as used in this study, with readings linearly related to the concentration of acetone extractable leaf chlorophyll per unit leaf area. This reading demonstrated that a

plant exposed to long days had greater leaf greenness, which is similar to the results found in purslane.

Nitrate reductase activity of leaves shows diurnal and seasonal changes, Nicholas et al. (1976) showed that the diurnal pattern in soybean leaves involved an increase in activity during the day and a decrease during the night; this may be a reason of why the growth in purslane is greater under long light exposure.

It is clear that there exists a direct effect of long day light exposure on the weight of the plant, especially the fresh weight of the leaves and the chlorophyll content (Novoa and Loomis 1981).

Comparing different purslane accessions provided the recognition of several characteristics specific for each specimen, even though the characterisation of approximately 40 varieties of purslane has not been described intensely (Matthews et al. 1993). Some differentiation was possible in this project as one wild accession from Canada was statistical significant different among the nine accessions used in this work. This wild accession from Sainte-Anne-de-Bellevue, Quebec, Canada had superior growth, despite the inclusion of several agronomic accessions including 'Golden' and 'Tall Green'.

This local wild plant developed long and reddish stems, with a mostly upright growth under controlled conditions, providing a favorable scenario for the production of

the plant with limited space, compared to the regular field habit with long prostrate stems.

Previous studies made on purslane have mostly used the agronomic accessions 'Golden' and 'Tall green' (Anastacio and Carvalho 2013, Franco et al. 2011, Palaniswamy et al. 1998, 2001, 2002, 2004, Teixeira and Carvalho 2009). Stem fresh weights of in those studies, were about 20 g higher than recorded in this project, due to harvest time. In the present study, plants were harvested and measured 51 days after sowing, while Palaniswamy et al. (2001 & 2004) measured the plants after 60 days of growth, or more. In a study on stress salinity in purslane, stem biomass was also relatively low (2.5 g), but harvest occurred after 20 days (Lara et al. 2011).

For making an accurate comparison between the results of this work and others using purslane, despite of its origin, the plants must be measured at the same time after been sown. For example, values of fresh weight of purslane for leaf and stem realized by Szalai (2010) and Fontana (2006) exhibit values similar to the ones found in this work, even though the varieties used in their work were wild accessions from Iran (Szalai 2010) and Spain (Fontana 2006).

From evaluating the agronomical potential of purslane, using a ratio of 50:50 for nitrate and ammonium, and photoperiods of 16 hours light, the growth of purslane represents a potentially viable crop. The use of wild accessions present in Quebec

represents an opportunity for entrepreneurial farmers to produce a plant with health and nutritive benefits.

5.2 Oxalic acid in purslane

The oxalic acid concentration in purslane leaves and stems was significantly influenced by the interaction of $N0_3$ -/ NH_4 +, photoperiod (light exposure) and accession. Studies on purslane for the concentration of oxalic acid have been done mostly comparing different ratios of $N0_3$ -/ NH_4 + (Fontana et al. 2006); other studies include the leaf stage as a main factor (Palaniswamy et al. 2004); however, studies such as this one, in which two ratios of $N0_3$ -/ NH_4 +, two photoperiods and nine accessions where carefully controlled throughout growth of the plants, have not been done before.

In this study, the difference in the concentration of oxalic acid among plants was clear, and the agronomical variety Accession 3, or Var. 'Golden' had the lowest oxalic acid concentration when grown under a ratio of 50:50 N0₃-/NH₄+ and 12 hours of light. When grown under these conditions, this accession was the only one that processed a suitable option for producing purslane with a low concentration of oxalic acid.

The growth of the plants, analyzed under the effect of NO_3^-/NH_4^+ and photoperiod, revealed that the plants grown under a photoperiod of 12 hours and a ratio of 50:50 NO_3^- / NH_4^+ had the lowest concentration of oxalic acid and are significantly different from plants grown under 16 hours light exposure and 25:75 NO_3^-/NH_4^+ .

Light exposure has been shown to have an effect on the concentration of oxalic acid in studies made on spinach (Proietti et al. 2004). Leaves of spinach grown under low light intensity had the lowest concentration of oxalic acid when compared to plants grown under high light intensity. This high concentration can be explain by the fact that oxalic acid can be degraded by oxalate oxidase (Loewus 1999), unlike previous assumption that oxalic acid was only an end product of metabolism with little turnover (Franceschi and Horner 1980). The activity of oxalate oxidase is increased under high light intensity, resulting in an increase in the portion of oxalate oxidase that can be degraded to oxalic acid (Proeitti et al. 2004). Under these conditions, it is possible that extended duration of time; the content of oxalic acid can be influenced by affecting the control of its degradation, rather than control during synthesis.

Other studies concerning the concentration of oxalic acid and light were made on Kalamegh or 'King of bitters' (*Andrographis paniculata*) (Palaniswamy 2005). That study revealed that plants exposed to bright light had 30% more concentration of oxalic acid than plants grown under soft light, suggesting a protective response against bright light in the photosynthetic apparatus. Previous studies demonstrated a correlation between increased light and the formation of oxalic acid in rhubarb leaves, and buckwheat (Pucher et al. 1939).

Franceschi and Horner (1980) inferred that the rise in the concentration of oxalic acid in plant tissues was due to new synthesis that may follow several major pathways;

such as, glycolate can be converted to oxalic acid and oxaloacetate also can be split to form oxalate and acetate, and I-ascorbic acid is also a precursor of oxalic acid.

Studies made on *Pistia statiotes* (water lettuceshowed the direct relationship between ascorbic acid and oxalic acid (Keates et al. 2000); identification of oxalic acid formation pathway in *Pistia stratiotes* revealed that L-galactose is a precursor of ascorbic acid and this in turn is further metabolized to oxalic acid. Other studies made on *Lemna minor* (common duckweed) also suggested a strong connection between ascorbic acid and oxalic acid. Saito (1996) pointed that L-ascorbate, which was formed from D-glucosone, was converted to oxalate, without showing any link with the pathway where glycolate and/or glyoxylate are precursors of oxalic acid.

The content of ascorbic acid has been shown to remain in low levels in plants that have been exposed to short periods of light (10-8 hours), these plants, once exposed to longer periods of light (14-16 hours) presented a several fold increase in the ascorbic acid content, which may lead to an increase in the quantity of oxalic acid by degradation (Nanda and Tayal 1976). The effect of nitrogen in the quantity of oxalic acid has been extensively documented, unlike the effect of photoperiod or light exposure.

Results of this work agree with the earlier reports on purslane (Palanyswamy et al. 2004, Fontana et al. 2006) and New Zealand spinach (Ahmed and Johnson 2000). The concentration of oxalic acid is affected by the intake of nitrogen both as NO_3^-/NH_4^+ . A combination of these two forms in a ratio of 50:50 appears to be beneficial in terms of

low oxalic acid concentration. Previous studies suggested that a N0₃/NH₄+ ratio of 25:75, 40:60 or 45:55 would offer plants with low oxalic acid in their leaves and stems, while in the present study, the comparison of two ratios (25:75 and 50:50) offered unequivocal results, indicating significant differences between the ratios studied and coherence with earlier reports.

When nitrogen is only provided as N0₃-, it has to be reduced in the shoots (nitrate reduction by nitrate reductase) to allow the plant to absorb the nitrogen. This reaction ends in the accumulation of several organic acids such as oxalic acid in leaves and stems (Libert and Franceschi 1987). Other theories refer the excess of oxalic acid in plants grown under N0₃- to high production of OH- ions during assimilation, the plant responds to the need to stabilize this ion excess by producing more oxalic acid (Pierce and Appleman 1943).

The third and last factor analyzed was the variation within accessions. The use of different accessions permitted the recognition of differences in oxalic acid concentration in purslane tissues. Significant difference in the oxalic acid concentration occurred amongst five accessions of Iranian purslane, with one accession, 'Borazjan', with the lowest concentration of oxalic acid (Gharneh and Hassandokht 2012). Difference in the concentration of oxalic acid between accessions can be expected.

5.3 Fatty acids in purslane

Previous studies with purslane showed that the nitrogen form had significant effect on the concentration of fatty acids, without altering the fatty acid composition (Fontana 2006, Palaniswamy 2000). In this work, it was evident that the nitrogen form, as well as the photoperiod and the accession, altered the concentration of fatty acids, specially the concentration of linolenic acid and α -linolenic acid in the leaves and stems of purslane. The concentration of ALA increased as much as 3- to 4-fold in the plants grown under 16 hours light and a N0₃//NH₄+ of 25:75 (Figure 3), this supports that the quantity of fatty acids can be altered under specific environmental and nutritional conditions (Fontana 2006, Liu et al. 2000, Kamal et al. 2012, Lara et al. 2011, Mortley et al. 20012, Palaniswamy 2000, 2001). Similarly, Szalai et al. (2010) reported plants grown under a N0₃//NH₄+ ratio of 25:75 had the highest concentration of ALA.

However, the concentration of LA in the present study was highest when plants were grown under a 50:50 ratio of $N0_3$ -/NH₄+ and 12 hours light, but under a $N0_3$ -/NH₄+ ratio of 25:75 and 16 hours light, LA concentration was 8% less (Figure 2).

Even though the results in this study demonstrate the influence of the NO_3^-/NH_4^+ form on the fatty acid concentration, these cannot be compared with the majority of studies made on purslane because the harvest was 70-80 days after sowing (Palaniswamy 2000, Mortley 2012). In the present study, the plants were harvested and

measured for their fatty acid concentration after 51 days of growth, a time that would have the minimum concentration of oxalic acid according to Palaniswamy et al. 2004.

Harvest times have been shown to have a significant influence on the concentration of fatty acids (Omara-Alwala et al. 1991, Ezekwe et al. 1999, Brown 2004, Mortley 2012). Plants harvested after 80 days of growth had almost double the amount of LA and ALA that plants harvest after 60 days of growth had (Mortley 2012).

However, when the quantity of fatty acids of plants harvested after 59 and 45 days were compared, there was no difference between the concentrations of fatty acids (Palaniswamy 2001). At this point, the study of photoperiod and accession combined with the nitrogen forms may offer an explanation. In this work, the interaction between these three factors was statistically significant. The reasons why the concentration of fatty acids increased at different nitrogen ratios and photoperiods remain unclear, expressing the need to conduct additional studies combining different nutritional and environmental characteristics on the growth of purslane and their effects on fatty acids.

Another important point worthy of mention is the ratio in which ALA and LA were found in the plants. Plants grown with a 12 hour photoperiod had the most suitable ratios of ALA and LA for human consumption (Figure 4). Accessions 1 and 3 grown under a 50:50 N0₃-/NH₄+ ratio had the nearest 1:1 ratio (Figure 4). These results are consistent with those of Omara-Alwala (1991) and Palaniswamy (2001), whom described significant differences in the ratio of ALA and LA when different nitrogen

forms and concentrations were studied. Even though the concentration of fatty acids, individually, are important for human nutrition, the ratio in which those are present, specially ALA and LA, are important issues in a balanced diet (Brown 2004, Ezekwe et al. 1999, Mortley 2012).

6.0 CONCLUSION

Combining different growth characteristic on purslane growth revealed desirable ways in which this plant can be produced. It was possible to determine how the nitrogen, photoperiod and accession affect the general growth of the plant, the concentration of oxalic acid, and the concentration of fatty acids (ALA and LA).

Plant growth was affected by the nitrogen form and photoperiod. Plant growth was greatest in general under the 16 hours light regiment. Accession 1 or 'local accession # 1' had the greatest growth value as compared with the other eight accessions. Accession 1 also had the highest values of LA and ALA. For LA, a nitrogen ratio 50:50 and 12 hour photoperiod resulted in the highest values. Those same conditions resulted in significant concentrations of ALA in Accession 1, although Accession 6 was superior.

Plants grown in 50:50 ratios of N0₃-/NH₄+ and received a 12 hour photoperiod had the lowest concentrations in oxalic acid. Accession 3 had the lowest value, but Accession 1 (found to have significant quantities of ALA and LA) also had similar low values of oxalic acid.

It was not possible to find a single accession grown under one set of environmental and nutritional conditions that displayed the highest growth, fatty acid concentration and oxalic acid concentration; but so far, Accession 1 represented the most desirable crop for human consumption, even though the conditions that offered the

highest quantity of fatty acid and the lowest concentration of oxalic acid are not the same conditions that offered the best growth of the plant.

The result of this work clearly showed that purslanes', growth under a $N0_3$ -/NH₄+ ratio of 50:50 and 12 hours light produced a desirable crop for consumption, offering the highest quantity of fatty acids and at the same time the lowest concentration of oxalic acid.

Accession No 1 (local accession from Quebec) offered the best option; with significant quantities of nutraceutical factors important in the dietary intake for health promotion and disease prevention, this shows that some wild plants can offer beneficial characteristics and may offer a suitable crop for local production.

Wild purslane as a crop in Canada is still a fledgling field of study; differences between the different local accessions in Canada remain unclear. Future studies should better elucidate the differences among these accessions and their nutraceutical characteristics.

7.0 TABLES

Table 4. Different accession of purslane acquired and their origin.

	Purslane accessions
1	Local accession # 1
2	Var. 'Tall Green'
3	Var. 'Golden'.
4	Saudi Arabia accession
5	Iran accession
6	Local accession #2
7	Local accession #3
8	Afghanistan accession
1	

9 Syria accession

Table 5: Composition (mg) of the nutrient solution used in the various fertilization treatments.

	N0 ₃ -/	′NH₄+
Compound	50:50	25:75
(NH ₄)(NO ₃)	205.6	78.9
(NH ₄)SO ₄	55.8	186.5
CaCl ₂	34.5	28.56
KCI	93.12	93.12
MgCl ₂	47.65	47.65
KH ₂ PO ₄	256	256

Table 6: The effects of N03-/NH4+ ratios combined with light exposures on plant growth variables amongst nine accessions of *Portulaca oleracea* (purslane).

	Light	Stem	Chl. Cont. #	Loof E\//+	Stom EW	Total EW/	
N03 ⁻ /NH4 ⁺	Exposure	Height	(SPAD		Stelli FW	TOLAT FVV	
	(hours)	(cm)	value)	(g)			
50:50	12	14.33†	27.68†	2.09†	1.64†	3.14†	0.09†
	16	16.59†•	34.95•†	4.81†•	2.92†•	7.73†•	0.16†•
25:75	12	13.21 ·	30.35+•	1.94•◊	1.35•	3.26•◊	0.08•◊
	16	12.34†•	31.31†•	3.32†•◊	1.64 ·	4.97 [†] ◊	0.12**
Significance		**	**	**	**	**	**

‡ Fresh weight.

+•◊ Within columns, values followed by the same symbol present statistical significant difference (P≥0.05) according to Bonferroni's multiple range test.

#Chlorophyll content

		Stem	Chl. Cont. #	Leaf	Stem	Total	Total
N0 ₃ -/NH ₄ +	Acc. ¹	Height	(SPAD	FW‡	FW	FW	DW⊀
		(cm)	value)		(g)	
50:50	1	22.19°	32.03 ª	5.33 d	3.83°	9.16°	0.15 ^b
	2	16.15 ^b	28.03 ^b	3.13 ^b	2.02 ^b	5.15ª	0.12 ^b
	3	9.30 ª	27.35 ^b	1.90ª	0.97 ^a	2.87 ª	0.13 ^b
	4	13.21 ^{a,b}	29.82 ^b	3.26 ^b	2.02 ^b	5.45 ^{a,b}	0.13 ^b
	5	9.33ª	30.82 ^{a,b}	3.00 ^b	1.30ª	4.17 ª	0.09ª
	6	17.60 ^b	33.18ª	4.12 ^{c,d}	2.77 ^{b,c}	6.23 ^b	0.11 ^b
	7	18.29 ^{b,c}	30.80 ^{a,b}	3.90 ^{c,d}	2.92 ^{b,c}	6.15 ^b	0.10 ^b
	8	15.82 ^b	32.47 ª	3.12 °	2.06 ^b	4.58 ª	0.15 ^b
	9	17.21 ^b	37.37 °	3.40 ^{c,d}	2.64 ^b	5.19ª	0.16 ^b
25:75	1	14.27 ^b	31.27 ª	2.53 ^b	1.54 ª	4.08 ª	0.13 ^b
	2	11.95 ^{a,b}	34.00 ª	1.63ª	1.06 ^a	^a 2.69 ^a	a0.09 a
	3	10.29ª	26.92 ^b	2.50 ^b	1.12ª	^a 3.62 ^a	^a 0.09 ^a
	4	10.97 ª	31.58ª	2.67 ^b	1.44 ^a	^a 4.11 ^a	^a 0.09 ^a
	5	9.48 ª	32.53 ª	2.34 ^b	1.08 ª	^a 3.27 ^a	^a 0.09 ^a
	6	16.68 ^b	30.75ª	2.99°	^a 1.93 ^a	^a 4.92 ^a	^b 0.13 ^b
	7	14.25 ^b	30.37 ª	3.15°	^a 1.68 ^a	^a 4.83 ^a	0.08 a
	8	13.78 ^b	30.73ª	2.83 ^b	^a 1.61 ^a	^a 4.44 ^a	^b 0.10 ^b
	9	13.31 ^b	29.37 ^b	3.08 ^b	2.03 ^b	^a 5.11 ^a	^b 0.13 ^b
Significance		**	**	**	**	**	**

Table 7: The effect of nitrogen ratio on plant growth variables amongst nine accessions of *Portulaca oleracea* (purslane).

¹ Accession (1) Local accession #1 (2) Var. 'Tall Green' (3) Var. 'Golden' (4) Saudi Arabia accession (5) Iran accession (6) Local accession #2 (7) Local accession #3 (8) Afghanistan accession and (9) Syria accession. ^{a,b,c} Within column, values with different letter denote significant difference, and those with the same letter do not represent a significant difference (P≥0.05) according to Bonferroni's multiple range test. # Chlorophyll content ‡ Fresh weight. ⊀ Dry weight. ** Significant at the 0.05 level of probability.

	Stem	Root	Chl. Cont. #	Leaf	Stem	Root	Total	Total
N03 ⁻ /NH4 ⁺	Height	length	(SPAD	FW‡	FW	FW	FW	DW≮
	(c	m)	Value)		(g)			
50:50	15.46	4.54†	31.32†	3.44	2.28	0.29	0.13	0.13
25:75	12.77	4.55†	30.83†	2.63	1.45	0.21	0.10	0.10
Significance	**	**	**	**	**	**	**	**

Table 8: Comparison of two different ratios of N0₃-/NH₄+ on *Portulaca oleracea* (purslane) growth.

‡ Fresh weight.

⊀ Dry weight

+ Values do not present significant difference within column (P≥0.05) according to Scheffe's multiple range tests.

Chlorophyll content.

Table 9: The effect of light duration on growth param	neters of <i>Portulaca oleracea</i> (purslane).

Light	Stem	Root	Chl. Cont. #	Leaf	Stem	Root	Total	Total
Exposure	Height	length	(SPAD	FW‡	FW	FW	FW	DW⊀
	(cm)		value)			(g)		
12	13.77	4.57†	29.02	2.02	1.5	0.14	3.2	0.08
16	14.46	4.52†	33.13	4.07	2.28	0.36	6.35	0.14
Significance	**	**	**	**	**	**	**	**

‡ Fresh weight.

⊀ Dry weight

+ Values do not present significant difference within columns (P≥0.05) according to Scheffe's multiple range test.

Chlorophyll content.

	Total FW‡			Total DW⊀		
ACCESSION	(g)			(g)		
1	6.61	±3.97 ª		0.13	±0.07 a	
2	3.92	±2.30 ^b		0.10	±0.04 ^b	
3	3.24	±1.52 ^b		0.10	±0.07 ^b	
4	4.77	±2.27 ^b		0.10	±0.04 ^b	
5	3.71	±1.71 ^b		0.09	±0.07 ^b	
6	5.57	±3.67 ª		0.12	±0.04ª	
7	5.49	±2.93 ª		0.09	±0.07 ^b	
8	4.51	±2.05 ^b		0.13	±0.04ª	
9	5.14	±0.07 ^b		0.15	±0.07 ª	
Significance	**			**		

Table 10: Relative growth response of nine *Portulaca oleracea* (purslane) culivars.

‡ Fresh weight.

⊀ Dry weight

¹ (1) Local accession #1 (2) Var. 'Tall Green' (3) Var. 'Golden' (4) Saudi Arabia accession (5) Iran accession (6) Local accession #2 (7) Local accession #3 (8) Afghanistan accession and (9) Syria accession.

^{a,b} Values within a column with different letter denote significant difference, and those with the same letter do not represent a significant difference ($P \ge 0.05$) according to Bonferroni's multiple range test.

	Stem Height		Chlorophyl	I Content	Root length		
	(cm)		(SPAD	value)	(cm)		
1	18.23	±5.25ª	31.65	±3.76ª	4.88	±1.61ª	
2	14.05	±5.64 ^b	31.02	±4.97 ª	4.35	±1.68 ª	
3	9.8	±5.63 °	27.13	±4.89 ^b	3.37	±1.72 ^b	
4	12.09	±5.51 ^b	30.7	±4.77 ª	3.9	±1.73 ^b	
5	9.41	±5.53 °	31.67	±5.39 ª	5.06	±1.74 ª	
6	17.15	±5.53 ª	31.97	±5.46 ª	5.04	±1.75ª	
7	16.27	±5.42 ª	30.59	±5.47 ª	5.37	±1.78℃	
8	14.8	±4.47 ^b	31.6	±5.35 ª	5.14	±1.55℃	
9	15.26	±3.47 ª	33.37	±5.29 ª	3.8	±1.23 ^b	
Significance	**		**		**		

Table 11: Variation in stem height, chlorophyll content and root length in nine culivars of *Portulaca oleracea* (purslane).

¹ (1) Local accession #1 (2) Var. 'Tall Green' (3) Var. 'Golden' (4) Saudi Arabia accession (5) Iran accession (6) Local accession #2 (7) Local accession #3 (8) Afghanistan accession and (9) Syria accession.

^{a,b,c} Within column, values with different letter denote significant difference, and those with the same letter do not represent a significant difference ($P \ge 0.05$) according to Bonferroni's multiple range test.

	Leaf FW‡		Stem	Stem FW		Root FW	
ACCESSION	(g)		(g	(g))	
1	3.93	±2.41 ª	2.69	±1.60 ª	0.28	±0.20 ^a	
2	2.38	±2.12 ^b	1.54	±1.21 ^b	0.19	±0.15 ^b	
3	2.20	±1.8 ^b	1.04	±1.01 ^b	0.14	±0.16 ^b	
4	2.96	±2.12 ^b	1.73	±0.72 ^b	0.28	±0.21 ^a	
5	2.61	±2.10 ^b	1.19	±0.91 ^b	0.21	±0.21 ^b	
6	3.55	±2.64 ª	2.35	±1.75ª	0.28	±0.21 ^a	
7	3.52	±2.67 ª	2.30	±1.78 ª	0.31	±0.21 ^a	
8	2.98	±2.36b	1.83	±1.23 ^b	0.28	±0.16 ^a	
9	3.24	±1.92 ª	2.33	±1.24 ª	0.28	±0.15 ª	
Significance	**		**		**		

Table 12: Variation in leaf fresh weight, stem fresh weight and root fresh weight in nine different accessions of *Portulaca oleracea* (purslane).

¹ (1)Local accession #1 (2) Var. 'Tall Green' (3) Var. 'Golden' (4) Saudi Arabia accession (5) Iran accession (6) Local accession #2 (7) Local accession #3 (8) Afghanistan accession and (9) Syria accession.

‡ Fresh weight.

^{a,b,} Within column, values with different letter denote significant difference, and those with the same letter do not represent a significant difference ($P \ge 0.05$) according to Bonferroni's multiple range test.
Table 13: Response of purslane to two N0₃-/NH₄+ ratios combined with two photoperiods in the concentration of fatty acids.

N03 ⁻ /NH4+	Light Exposure	¹ C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	
-	(hours)	Percentage (%)							
50:50	12	9.94	9.20†	0.48	1.60	3.60	16.00†	24.55†	
	16	8.79	9.85 ·	0.02	1.04	2.54	13.97 ·	30.77+•	
25:75	12	8.50	9.60	0.50	2.66	5.22	10.79**	16.25**	
	16	10.99	13.21**	1.61	1.33	4.48	17.84•◊	34.36**	
Significance			**				**	**	

¹Myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and α -linolenic acid (18:3).

+•◊ Within a column, values follow by the same symbol present statistical significant difference (P≥0.05) according to Bonferroni's multiple range test according to Bonferroni's multiple range test.

** Significant at the 0.05 level of probability.

Table 14: Fatty acid concentration in leaves and stems of purslane grown under $N0_3$ -/NH₄+ ratio of 50:50 and 25:75

NO ₆ -/NH /+	¹ C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	
1103711114	Percentage (%)							
50:50	9.36	9.52 [†]	0.25	1.32	3.07	14.98 [†]	27.66 [†]	
25:75	9.74	11.40 [†]	1.05	1.99	4.85	14.31 [†]	25.37 [†]	
Significance		**				**	**	

¹Myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and α -linolenic acid (18:3).

+ Values presented statistical significant difference within columns (P≥0.05)

according to Scheffe's multiple range test.

** Significant at the 0.05 level of probability.

Table 15: Fatty acid concentration in leaves and stems of purslane grown under two photoperiods.

Light Exposure	¹ C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
(Hours)			Pei	rcentage	(%)		
12	9.22	9.39†	0.49	2.13	4.41	13.39 [†]	20.40†
16	9.89	11.52†	0.82	1.18	3.51	15.90†	32.56†
Significance		**				**	**

¹ Myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and α -linolenic acid (18:3). ** Significant at the 0.05 level of probability.

+ Values presented statistical significant difference within columns (P≥0.05) according to Scheffe's multiple range test.

Table 16: Effect of N0₃-/NH₄+ ratio combined with two photoperiods on the oxalic acid content in leaves and stems of purslane.

	Oxalic acid content mg/100 g FW				
	Light exposure				
N03 ⁻ /NH4 ⁺	Hours				
	12	16			
50:50	367.58	609.17†			
25:75	463.43	612.87†			
Significance	**	**			

+ Values do not represented a statistical significant difference (P≥0.05) according to Bonferroni's multiple range test.

** Significant at the 0.05 level of probability

Table 17: Left: Oxalic acid content in purslane leaves and stems grown under $N0_3$ -/NH₄+ ratios of 50:50 and 25:75. Right: Oxalic acid content (mg/100g FW) on purslane leaves and stems grown under 12 and 16 hours light exposure.

N0 ₃ -/NH ₄ +	Oxalic acid	Light Exposure	Oxalic acid	
	mg/100g FW	(Hours)	mg/100g FW	
50:50	488.36†	12	415.5 [†]	
25:75	538.15 [†]	16	611.01†	
Significance	**		**	

+Statistical significant difference within columns from the same group (P≥0.05) according to Scheffe's multiple range test.

** Significant at the 0.05 level of probability

A accession 1	Oxalic acid				
Accession	mg/100g FW				
1	547.6	±221.92 b			
2	659.04	±165.69 ª			
3	412.07	±250.11 °			
4	478.7	±195.10 ^{b,c}			
5	528.3	±189.62 ^{b,d}			
6	430.94	±284.31 °			
7	570.74	±160.22 d			
8	520.84	±148.20 ^{b,d}			
9	471.07	±168.55 ^{b,c}			
Significance	**				

Table 18: Oxalic acid content from leaves and stems of nine accessions of purslane.

¹ (1) Local accession #1 (2) Var. 'Tall Green', (3) Var. 'Golden' (4) Saudi Arabia accession (5) Iran accession (6) Local accession #2 (7) Local accession #3 (8) Afghanistan accession and (9) Syria accession.

^{a,b,c,d} Within column, values with different letter denote significant difference, and those with the same letter do not represent a significant difference ($P \ge 0.05$) according to Bonferroni's multiple range test.

** Significant at the 0.05 level of probability







Values inside the same circle on the same Cartesian plain do not present statistical significant difference; circles with different dashes present statistical significant difference within and between Cartesian plains with $P \ge 0.05$ according to Bonferroni's multiple range test.



N03:NH4+25:75





Values inside the same circle on the same Cartesian plain do not present statistical significant difference; circles with different dashes present statistical significant difference within and between Cartesian plains with $P \ge 0.05$ according to Bonferroni's multiple range test.



C18:2 C18:3

Figure 4: Ratio of linoleic (C18:2) and α -linolenic (C18:3) acids concentration in percentage (%) among nine accession of purslane ^a(1) Local accession #1 (2) Var. 'Tall Green' (3) Var. 'Golden' (4) Saudi Arabia accession (5) Iran accession (6) Local accession #2 (7) Local accession #3 (8) Afghanistan accession and (9) Syria accession, when grown under a N03-/NH4+ ratio of 50:50 (right) and 25:75 (left), each one with photoperiods of 12 and 16 hours light.



▲ C14:0 • C16:0 + C16:1 × C18:0 ● C18:1 ◆ C18:2 ■ C18:3 ^b

Figure 5: Concentration of fatty acids among nine accessions of purslane ^a(1) Local accession #1 (2) Var. 'Tall Green' (3) Var. 'Golden' (4) Saudi Arabia accession (5) Iran accession (6) Local accession #2 (7) Local accession #3 (8) Afghanistan accession and (9) Syria accession.

^b Myristic acid(C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and α -linolenic acid (18:3) for each of the nine accessions:





Figure 6: Oxalic acid content in the nine accessions of purslane grown under 12 and 16 hours light and N0₃:NH₄+ ratios of 50:50 and 25:75. (1) Local accession #1 (2) Var. 'Tall Green' (3) Var. 'Golden' (4) Saudi Arabia accession (5) Iran accession (6) Local accession #2 (7) Local accession #3 (8) Afghanistan accession and (9) Syria accession.

Values inside the same circle on the same Cartesian plain do not present statistical significant difference; circles with different dashes present statistical significant difference within and between Cartesian plains with P≥0.05 according to Bonferroni's multiple range test.



Figure 7: Druse-shaped calcium oxalate crystals from *Portulaca oleracea*. Light microscopy photographs of stained crystals on distal vascular leaf tissues. Scale bar = 25µm, 40X magnification.



Figure 8: Calcium oxalate druses surrounded the mid vein in *Portulaca oleracea*. Crystals' appearance and arrangement of single druses. Scale bar =50 μ m, 20x magnification.



Figure 9: Idioblast cell containing druse-shape calcium oxalate crystals in young leaf tissue of *Portulaca oleracea*. Light microscopy (scale bar = $50 \mu m$, 20X magnification).



Figure 10: Population of calcium oxalate crystals in the vascular tissue and palisade layer of *Portulaca oleracea*. Light microscopy (scale bar=100µm, 10X magnification).



Figure 11. Morphological differences of calcium oxalate druses-shapes crystal's in *Portulaca oleracea*. A) Typical stellate calcium oxalate crystal. B) Calcium oxalate crystal with unclear shape. Crystal size varies between 25 and 50 μ m. Light microscopy (scale bar = 50 μ m, 20X magnification).

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