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Full Length Research Paper

Effect of ambient gases and soil moisture regimes on carbohydrate translocation in kidneybean plants grown in pots in Riyadh, KSA

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This study designated to examine the effect of elevated gases in four localities of Riyadh City on carbohydrate for parts of kidneybean plants (*Phaseolous vulgaris* L.) grown in pots under two soil moisture regimes (well-watered vs. restricted water). Carbohydrate analysis results showed increases in kidneybean samples under well-watered conditions compared to restricted soil moisture. Most kidneybean samples at Embasses site exhibited higher soluble, insoluble and total carbohydrate concentrations while the Batha site samples have lower values of these fractions. Batha site reduced the flux of carbohydrates from source to the sinks of both soil moisture regimes. This study concluded that there was a good relation between the effect of highly polluted localities and kidneybean leaves carbohydrate content and its translocation.

Key words: Gas pollution, Riyadh, kidneybean, carbohydrate content.

INTRODUCTION

Air pollution is a problem that depends mainly on total mass of pollutants emitted into atmosphere together with the atmosphere conditions that affect their fates and transport. As population, urban centers, and industries have grown, an increasing number of reports have appeared during the past 25 years regarding O_3-induced foliar injury on sensitive plants in many countries including KSA, Australia, Austria, Belgium, Canada, France, Germany, Greece, India, Israel, Italy, Japan, Mexico, the Netherlands, Pakistan, Peoples Republic of China, Poland, Russia, Spain, Sweden, Switzerland, Taiwan, United Kingdom, and Ukraine (Krupa et al., 1998).

Atmospheric ozone (O_3), nitrogen dioxide (NO_2) and sulphur dioxide (SO_2) are part and parcel of global climate change. Although ozone at the ground level is a "greenhouse gas," it plays a minor role in regulating our air temperature, contributing only about 7% to the total warming effect (Krupa, 1997). There is also a naturally occurring beneficial O_3 layer in the upper atmosphere (between 15 and 50 km above the surface) that strongly absorbs harmful ultraviolet radiation (about 210 to 290 nm: radiation <280 nm is UV-C and 280 to 315 nm is UV-B). In contrast, there are both natural and human-made sources of O_3 at ground level. Because of these natural sources, there is a background average O_3 concentration of roughly 20 to 30 nl/liter (ppb) everywhere (Finlayson-Pitts and Pitts, 1999). It is highly questionable whether there is any place on earth that has not been influenced by modern day human activity, and therefore background values will vary with location. Natural sources consist of lightning during thunderstorms and downward intrusions of O_3 from the upper atmosphere.

The most impact of O_3 was shown to be affected by level of moisture stress but not by SO_2. Chronic exposure of "Essex" soybean to 6 (nL L^{-1}) O_3 for 8 h day^{-1}, 5 day $week^{-1}$, for 18 week in the greenhouse caused a 34% reduction in yield compared to charcoal-filtered air. Sulfur dioxide in combination with O_3 and NO_2 caused no additional reduction in yield, but lower dosages of SO_2 increased yields compared to the O_3 treatment, apparently by retarding O_3 induced premature senescence. Emissions from a power plant had no adverse effect on yield on the cultivar Essex during a 3 year field study (1981 - 1983) (Jones et al., 1985).

Soluble carbohydrates were affected by chronic O_3 exposure. Miller (1989) indicated that O_3 tended to reduce soluble carbohydrates and starch in leaves, stems and roots. Also, positive interaction effects between the growth stage of plants and the effects of O_3 on the concentration and partitioning of reducing sugars, sucrose, and starch in plant tissues were reported. Unsworth et al. (1984), on soybean, noted that O_3 caused seed yield losses which were related to the reduction in leaf area (LA) and leaf area duration (LAD). In addition to the reduction in LA (less photosynthetic leaf tissue) with increasing O_3 concentration, leaves became less efficient in converting atmospheric CO_2 into seed yield as was suggested by the reduced seed yield to LAD ratio.

Ozone also affects indirectly on carbohydrates by its effect on photosynthesis and respiration, independently of any direct effect that O_3 may have on enzymes that regulate the metabolism and translocation of carbohydrates (Runeckles and Chevone, 1992). Tingey (1974) reported that *Pinus ponderosa* exposed to chronic O_3 concentrations (control and 100 nL L^{-1} O_3) had reduced leaf soluble carbohydrates during the first month of fumigation but increased concentrations during the following months (2 to 5 months) and suggested that the retention of carbohydrates in the leaves could cause reduction in photosynthesis by feedback inhibition and reduces the amount of assimilates for translocation to sinks within the plant. Slaughter (1987) exposed wheat plants to a range of O_3 concentrations (CF, NF, NF + 20, NF + 40 and NF + 80 nL L^{-1} O_3) from anthesis until harvest and found a small decrease (not significant) in total soluble carbohydrates content for seeds from O_3 stressed plants. Mulchi et al. (1992) exposed soybean plants to chronic O_3 stress (43 and 66 nL L^{-1} O_3) and observed that starch concentrations in leaves decreased 30% and doubled for the 40 and 66 nL L^{-1} O_3 treatments, respectively. They suggested that plants grown under increased O_3 stress (66 nL L^{-1} O_3) were limited by the ability to export carbohydrates to sinks such as nodules and pods but individual sugars (i.e. glucose, fructose and sucrose) were not significantly affected by O_3 stress. Pausch et al. (1996) found that O_3 stress on soybean caused a retention of ^{13}C labeled photosynthate products in leaves with less transport to sinks such as root and pods. Variation in carbohydrate response to O_3 stress may reflect difference in O_3 concentrations, growth stages, species, cultivars within species, etc. Also, O_3 tolerance has been associated with several biochemical characteristics such as higher concentrations of reducing sugars (Heck, 1990) which may react with the free radicals produced by O_3 and help to overcome the phytotoxic effect of O_3 (Malhotra and Khan, 1984).

The partitioning, or more correctly, the translocation of the non-structural carbohydrates (e.g. starch, glucose, sucrose, fructose) to vegetative, root and reproductive organs, ultimately affects the proportion of dry matter accumulated (Cooley and Manning, 1987). Observations indicate that carbohydrate translocation patterns in plants are altered by O_3. Gorrissen et al. (1991) reported that O_3 stressed douglas fir (*Pseudotsuga menziesii*) seedlings that were allowed to take up ^{14}C exhibited decreased release of ^{14}C in root/soil compartment during the first few days following treatments. However, weeks after O_3 treatments, the soil respiration activities were similar in all treatments, indicating that trees were recovering from stress. Loblolly pine seedlings, which were treated with CF air or 120 nL L^{-1} O_3 for 12 weeks, and labeled with ^{11}C, had altered ^{11}C partitioning (Spence et al., 1990). The high O_3 exposure reduced the speed of phloem transport by 11%, phloem photosynthate concentration by 40%, and total carbon transport to roots by 45%, similar to observations by Pausch et al. (1996) for soybean.

This study will focus on the influence of gases pollution exposure on carbohydrates fractions for parts of kidney-bean (*Phaseolous vulgaris* L.) plant grown in pots at different localities in Riyadh city, KSA.

MATERIALS AND METHODS

Experimental

This study concerned the short-term impact of air quality treatments and two soil moisture regimes on plant carbohydrates and nitrogen contents. Kidneybean seed plants (*Phaseolous vulgaris* L.) were first grown in pots at the beginning of January for 20 days at the Botany and Microbiology Department, Faculty of Science, King Saud University (KSU). Then, 3 replicates of pots were kept at the same site (KSU), others groups were transferred to Embasses, Batha, and Al-Naseem sites. Two moisture regimes were included for all localities.

Gases measurements

Air temperature, rain fall, relative humidity and wind speed were recoded in Table 1. Monthly concentrations of ambient O_3, SO_2 and NO_x were measured using multi-gas analyzer (Gray Wolf, Sweden).

Sampling

Plant organs were removed from two plants from all pots under the different treatments three times during vegetative and reproductive growth: one before flowering (leaves); the second at early pod fill or pre-grain fill (leaves); and the third at grain fill (leaves, roots and nodules). The leaf samples for kidneybean were separated into upper, middle and lower canopy position. Samples were temporarily stored at – 40°C until freeze-dried. The freeze-dried samples were grounded finely using a Wiley mill equipped with a 60-mesh screen.

Determination of carbohydrates fractions

For soluble carbohydrates, grounded samples were weighted (50 mg) and combined with 5 mL of hot deionized water and homo-genized for 1 min with a polytron blender. The homogenate was incubated for 30 min in a hot water bath (90°C) to halt the enzyme activity. Total carbohydrates were obtained in grounded samples using 1% amylo-glucosidase enzyme in 0.2 M acetate buffer, pH

Table 1. Mean values of meteorological parameters at Riyadh city, KSA.

Month	Air temperature (°C)	Humidity (%)	Wind velocity(km/hr)	Rain-fall (mm)
January	18	44	7	18.8
February	30	43	8	12.6
March	37	40	7	13.6
April	40	32	8	1.78
May	43	28	6	0.55
LSD ($p \leq 0.05$)	3.8	12.6	1.2	4.7

4.45 at 45°C for two days and measured as soluble sugars (Latzko and Gibbs, 1974). The solutions from soluble and total carbohy-drates were filtered through glass fiber filter discs and the volumes adjusted to 10 mL. The crude extracts (1 mL) were diluted in 10 mL of deionized water prior to carbohydrate analyses. Slaughter and Livingston (1993) performed carbohydrate analyses using the Dionex 4000 series Bio LC carbohy-drates system (Dionex, Sunny-vale, CA) following the procedure described. Starch concentrations (mg/g) were calculated by subtrac-ting soluble carbohydrates (mg/g) from total carbohydrates (mg/g).

Statistical analysis

Statistical analyses were carried out using the SPSS BASE 10.0 (SPSS Inc., Chicago, IL) packages. Data were tested by ANOVA and F-protected LSD separated means at $p \leq 0.05$ levels.

RESULTS

Variations in climate and gases

Mean values of meteorological parameters at Riyadh city, KSA were listed in Table 1. Changes in mean con-centrations of O_3, SO_2 and NO_2 during the growing of kidneybean (*Phaseolous vulgaris* L.) at industrial city, Riyadh, KSA are listed in Table 2. Mean monthly concen-trations of O_3 gradually increased in summer reaching to 110 nL L^{-1} in May and recording the lowest concentration in January being 40 nL L^{-1}. Also, gradual increase in SO_2 and NO_2 concentrations was observed. High values were recorded during hot months while cool months were vice versa. High SO_2 and NO_2 levels reached 35 and 33 nL L^{-1}, respectively, while low levels are 15 and 21 nL L^{-1}, respectively.

Carbohydrate fractions

Leaves

The effects of gases pollution and moisture regimes on total soluble sugars for kidneybean leaves at three growth stages of plant development (i.e. flowering, early pod, and grain-fill) are illustrated in Table 3. Moisture regimes produced significant effects in the middle cano-py position at flowering stage and in the upper canopy position at early pod stage of development with lower sugar levels under dry treatments. Localities treatments caused significant difference in soluble sugar levels at all three canopy positions showing higher soluble sugar levels at Batha site. Also, the upper canopy position showed much higher levels than leaves lower in the canopy. The effects of KSU site on soluble sugar contents in leaves of kidneybean were non-significant in all cases.

Insoluble sugar contents in kidneybean leaves under the four localities air quality and two moisture regimes are listed in Table 4. The effect of soil moisture on starch accumulation in kidneybean leaves showed significant increases in wet conditions in all growth stages, espe-cially in upper leaves. Embasses site increased the starch accumulation in leaves while Batha and Al-Naseem sites leaves exhibited reductions in insoluble carbohydrate levels. Leaves under high gases treatments (Batha and Al-Naseem sites) typically showed lower levels of insoluble carbohydrates than carbon-filtered controls with only two instances where the difference were significant, mid-canopy samples. The KSU and Embasses sites generally increased the insoluble carbo-hydrate levels in upper canopy leaves, especially under high moisture concentrations. The effects of moisture levels on insoluble sugar contents were observed to be varied.

The effects of NO_2, O_3, SO_2 and soil moisture levels on the total leaf carbohydrate concentrations are summarized in Table 5. Leaf carbohydrate levels in the upper canopy were lower under dry conditions during all three-growth stages but higher under dry conditions in the lower canopy position, especially during grain-fill. With regard to four localities treatments, exposure to KSU and Embasses sites increased the total carbohy-drate contents compared to Batha and Al-Naseem sites. The highest concentrations of total carbohydrates for all growth stages were observed during early pod develop-ment. With respect to the combination of elevated gases, total carbohydrate levels at all three canopy positions were comparable if not slightly larger than were observed in the KSU and Embasses sites treatments.

Roots and nodules

Table 2. Mean values of gases concentration (nL L^{-1}) at four localities, Riyadh, KSA during the growth period of kidneybean.

Month	O_3 (nL L^{-1})	SO_2 (nL L^{-1})	NO_2 (nL L^{-1})
KSU			
January	32	17	12
February	25	12	12
March	22	11	13
April	43	16	14
May	46	22	16
Embasses site			
January	31	15	14
February	26	11	11
March	27	11	11
April	33	12	11
May	39	15	14
Batha site			
January	45	22	23
February	41	18	17
March	67	16	28
April	77	25	33
May	112	33	38
Al-Naseem site			
January	40	15	21
February	52	20	24
March	77	25	25
April	89	33	35
May	110	35	33

KSU = King Saud University site.

Table 3. Mean values for soluble sugars (mg g^{-1}) for kidneybean leaves from pots under atmospheric gases enrichments, and soil moisture regimes at four localities, Riyadh, KSA.

Treatment	Canopy position		
	Upper	Middle	Lower
Moisture means			
Wet	31.5	23.4	34.7
Dry	25.7	21.7	19.5
Statistical Sign	***	NS	***
Localities means			
KSU	20.7	13.7	7.7
Embasses site	25.7	14.4	8.8
Batha site	13.5	9.9	5.6
Al-Naseem site	14.5	10.3	6.7
$LSD_{(P \leq 0.05)}$	3.8	5.3	4.8

*$P \leq 0.1$; **$P \leq 0.05$; ***$P \leq 0.01$.
KSU = King Saud University site.

Table 4. Mean values for insoluble sugars (mg g^{-1}) for kidneybean leaves from pots under atmospheric gases enrichments, and soil moisture regimes at four localities, Riyadh, KSA.

Treatment	Canopy position		
	Upper	**Middle**	**Lower**
Moisture means			
Wet	22.5	22.4	12.2
Dry	19.1	21.1	6.1
Statistical sign	*	NS	***
Localities means			
KSU	30.1	19.2	8.7
Embasses site	28.3	22.1	8.1
Batha site	18.1	19.6	4.3
Al-Naseem site	19.0	13.3	5.2
$LSD_{(P \leq 0.05)}$	4.1	3.7	3.4

*P ≤ 0.1; **P ≤ 0.05; ***P ≤ 0.01.
KSU = King Saud University site.

Table 5. Mean values for total sugars (mg g^{-1}) for kidneybean leaves from pots under atmospheric gases enrichments, and soil moisture regimes at four localities, Riyadh, KSA.

Treatment	Canopy position		
	Upper	**Middle**	**Lower**
Moisture means			
Wet	42.7	42.1	32.2
Dry	36.5	41.1	26.1
Statistical sign	*	NS	*
Localities means			
KSU	47.5	49.4	38.1
Embasses site	48.5	50.6	38.1
Batha site	32.4	39.2	24.1
Al-Naseem site	33.5	33.9	25.0
$LSD_{(P \leq 0.05)}$	3.4	3.1	1.1

*P ≤ 0.1; **P ≤ 0.05; ***P ≤ 0.01.
KSU = King Saud University site.

bean roots and nodules are summarized in Table 6. In term of air quality treatment effects, the results were typically significant for KSU and Embasses sites showing increases in both the soluble and the total carbohydrates in the roots and the nodules. The effects of Batha and Al-Naseem sites treatments were generally non-significant compared to the KSU and Embasses sites with slightly lower carbohydrate levels being found in the roots and the nodules. Embasses sites treatments showed increase in soluble and total carbohydrate levels for roots and especially under the dry moisture regimes.

DISCUSSION

In a typical urban atmosphere like Riyadh, O_3, SO_2 and NO_2 concentrations increase rapidly between 1200 and 1500 h entire the day light and when the intensity of solar radiation is at a maximum in hot months and when the NO_2:NO ratio is large (Krupa et al., 2001). The rate of O_3 formation may then decline, reaching a steady state during the late afternoon to early evening hours. After that period, O_3 concentrations fall as NO_2 breakdown diminishes and as fresh emissions of NO deplete the O_3. This daily pattern is quite different at high elevations (in general, above approximately 1,500 m from the surface or above the so-called mixed layer of the atmosphere), where O_3 concentrations remain relatively steady through day and night. At that altitude, there is an O_3 reservoir, and destruction of that O_3 by the surface is insufficient to produce the type of daily patterns observed at lower elevations (Krupa et al., 2001). They also reported that

Table 6. Mean values for soluble, insoluble and total sugars (mg g^{-1}) for kidneybean nodules and roots from pots under atmospheric gases enrichments, and soil moisture regimes at four localities, Riyadh, KSA.

Treatment	Nodules			Roots		
	Soluble	Insoluble	Total	Soluble	Insoluble	Total
Moisture means						
Wet	25.7	18.1	43.8	22.7	22.1	44.8
Dry	23.2	14.5	37.7	16.5	21.1	37.6
Statistical sign	NS	*	***	**	NS	**
Localities means						
KSU	39.1	45.5	84.6	22.5	19.2	41.7
Embasses site	41.2	46.1	87.3	24.5	20.2	44.7
Batha site	27.1	24.2	51.3	12.1	14.5	26.6
Al-Naseem site	31.1	23.1	54.2	13.1	12.4	25.5
$LSD_{(P \le 0.05)}$	4.1	2.2	2.5	2.1	3.8	5.1

*$P \le 0.1$; **$P \le 0.05$; ***$P \le 0.01$.
KSU = King Saud University site.

high levels of SO_2 and NO_2 concentrations cannot produce more damage in presence of high O_3.

The carbohydrate results revealed that moisture regimes produced only significant effects on the soluble carbohydrates in the middle canopy position at the flowering stage and in the upper canopy position at early pod stage of the kidneybean development with lower sugar levels under dry treatments. Also, there were mixed effects for the soil moisture on insoluble and total carbohydrate concentrations. Miller et al. (1995) reported that impacts of water deficit on carbohydrate levels were less consistent. As kidneybean leaves develop and age, their carbohydrate status changes. The data showed continuous increases in the leaf carbohydrate levels during flowering and early pod-fill and decreases during the grain-fill stage. Similar results were obtained by Giaquinta et al. (1981). The decreases in carbohydrate concentrations were due to translocation of sugars from the leaf to the seed samples throughout the seed filling.

In all patterns, Embasses site treatments increased the soluble, the insoluble and the total carbohydrate levels in the kidneybean leaves, the roots and the nodules. Rowland-Bamford et al. (1996) and Balaguer et al. (1995) reported that elevated CO_2 resulted in an increase in the carbohydrate fraction concentrations. In most cases, decreases in the concentration of sugars were found in different organs of kidneybean with high O_3 (NF + O_3) which agrees with previous work by Andersen and Rygiewicz (1998), Miller et al. (1995), Balaguer et al. (1995), Kostka-Rick and Manning (1992), Mulchi et al. (1992) and Amundson et al. (1991). Results obtained by Kostka-Rick and Manning (1992) and Mulchi et al. (1992) have shown that elevated O_3 reduced the flux of carbohydrates from source (leaves) to the sinks (shoots, roots, nodules and grains) of kidneybean.

The results in this paper agree with that of Balaguer et al. (1995). Interactions between the air quality treatments and soil moisture regimes occurred infrequently, although restricted water reduced the negative effects of O_3 for soluble sugar levels and starch concentrations for higher and lower canopy position of kidneybean leaves. In such cases, it is likely that stomatal conductance was reduced thereby reducing CO_2 uptake for photosynthesis by the leaves (Krupa and Kickert, 1989 ; Chernikova, 1998). Ozone independently effects enzymes that regulate the metabolism and translocation of carbohydrates (Runeckles and Chevone, 1992) while Tingey (1974) suggested that the retention of carbohydrates in leaves could cause reduction in photosysnthesis by feedback inhibition and reduce the amount of assimilates for translocation to sinks within the plant. Variation in carbohydrate response to O_3 stress may reflect difference in O_3 concentrations growth stages, species, cultivars within species, etc. Also, O_3 tolerance has been associated with several biochemical characteristics such as higher concentrations of reducing sugars (Heck, 1990) which may react with the free radicals produced by O_3 and help to overcome the phytotoxic effect of O_3 (Malhotra and Khan, 1984).

Conclusion

It is evident from this research that the flow of the carbohydrates from the source to the sinks are affected by increased levels of studied gases. This flow provides evidence towards a mechanism of O_3 action that involve short-term flux inhibition of C products from the leaves to the other organs including below-ground roots, nodules and microbial community which also depend on C fixed by photosynthesis.

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