Competition Indicator in Alfalfa Populations Following Recurrent Selection to Glyphosate Tolerance

M. Abd El-Sattar Ahmed[1*], Ahlam, H. Mostafa[2], Thanaa. I. Milad[3], and T.A. Mahmoud[4]

1 Professor, Crop Science Dept., Fac.Agric. (El-Shatby), Alexandria university (**Mohamed.A@alexu.edu.eg**)
2 Researcher, forage crops section, ARC, Nubaria, Egypt.
3 Professor, Crop Science Dept., Fac.Agric. (El-Shatby), Alexandria university. (**s.i.milad@alexu.edu.eg**)
4 Head Research,forage crops section, ARC, Nubaria, Egypt.
*; corresponding author.

ABSTRACT

The bad cide in adopting cide-tolerant alfalfa cultivar is the shift in weed communities with rise proportion of tolerant species. The main objective of the recent study was to trace competition indicator of alfalfa populations as affected by recurrent selection to glyphosate tolerance. Two cycles of recurrent selection for Glyphosate tolerance were imposed on each base population. Competition indicators following selection included plant characters and weed bioassay. In most studied populations, plant height decreased with the first cycle of selection to glyphosate tolerance by -8.991, -3.225, -1.240 and -0.426% in *Siwa*, *Hasawi*, C.U.F101 and *Baladi*1 populations, respectively. Except for, *Sirivar* population an increase by +6.740 % was accompanied with selection to glyphosate tolerance. On the other hand, by cycle two of selection, populations had different response relative to C1, where Baladi1, C.U.F101 and *Sirivar* populations had a decrease in plant height with values reached -1.761, -0.720 and 0.617 cm, respectively. While, *Hasawi* and *Siwa* populations showed an increased plant height by +3.816 and +3.197cm, respectively. Glyphosate treated plots (+ and -) were evaluated by weed bioassay in terms of green and dry weight during a course of three successive cuttings. Exposed genetic materials to glyphosate treatments were selection cycles (C_0, C_1 and C_2) of the five studied populations.The first cycle of selection to glyphosate tolerance expressed a significant increase of 9.59 and 10.93% relative to the base population as green and dry weed weights, when evaluated under glyphosate treating condition. The second cycle of selection was associated with a reduction of 29.63 and 28.88% in green and dry weight of weed. This might indicate a proliferation of alfalfa plants that were selected for glyphosate tolerance, the matter that reduced the mass of weeds, whether, as green or dry weight.

Keywords: Competition, Alfalfa,Populations, Recurrent Selection, Glyphosate tolerance, Tillering, Plant height, Weed bioassay.

INTRODUCTION

Alfalfa "*Medicago sativa*, L." that has large genetic diversity, is among the most important forage crops "king of forages". That provided alfalfa genotypes to occupy different environments. The importance of alfalfa forage goes to its high content of protein and minerals, besides, high degree of palatability. Volunteer weeds in alfalfa fields are mostly of lower quality and palatability, especially "*Urospermum picriods*" and "*Xanthium spinosus*". That affect the value and persistence of alfalfa fields.

The early seedling stage of alfalfa is the most vulnerable to weed competition. Weed hazards extend up to the third cutting of the establishment year. Selective herbicides as a control measure in alfalfa fields were used very little. That goes to its high price, limited effectiveness and herbicidal injury. Glyphosate is a systemic non-selective foliar applied herbicide. Irrespective of glyphosate non- selectivity, several plant species exhibit levels of tolerance to its effect (Gottrup, *et al.*,1976), reductions to sorption and limited translocation from vegetative to reproductive organs (Neal *et al.*,1985). Several trials have been made to select a glyphosate tolerance genotypes *in vitro*. In each of them tolerance was due to an increase in 5-enolpyruvy shikimate 3-phosphate synthase (EPSPS) activity (Shah *et al.* 1986).

The registration of the new alfalfa variety Roundup Ready ® started by mid-2005. The new variety came-up after the translocation of resistance- give to alfalfa germplasm. That enable the new plant to resist the effects of general eradication herbicide "glyphosate". The good consequences of adopting such new type of cultivars includes an improvement in yield, quality and turn- over. Also, glyphosate is a short- durated herbicide in soil, with very limited influences on soil Flora and Fauna, besides, salt- effect on mammals. The bad cide in adopting cide-tolerant alfalfa cultivar is the shift in weed communities with rise proportion of tolerant species. Miller *et al.* (2006), summarized the benefits of using Rundup Ready ® alfalfa in North states as ; 1) better control of noxious- weed species, 2) insure less botanical injury- indicators, 3) Provide flexible management practice regarding the time of application, prevailing weather, 4) reduce weed competition allowing for better establishment and good forage quality, 5) ensure the use of pre-tested safe- herbicide, and 6) secure high quality hay free- from noxious weeds. The expected increase in yield with this new technology reached about 1.4 to 3.3 ton. $acre^{-1}$ Late application of glyphosate to alfalfa Fields caused a reduction in yield reached 0.3 to 0.8 ton. ha^{1}. Orloff and Putnam (2011). Discussed the good and bad consequences of used roundup ready alfalfa cultivar. Growers of alfalfa at majority were satisfied with good consequences of new cultivar. They related their acceptance to this new cultivar to good limitation of weed competition, flexible application, and potentiality of eliminating bad weeds. They also reported a higher forage yield and better-quality forage. Green, (2012), explained the merits of relaying on glyphosate resistant crops rather than changing the used herbicide seeking for more efficient weed control system. The merits of the first strategy depend on using single herbicide (glyphosate), which is common to farmers with easy, effective, economic and environmentally friendly sides. He added that, the efficiency of glyphosate in eradicating weeds is not equal to all species, consequently some species acquire resistance, driving the control system relaying on glyphosate resistance to be unsustainable. He advised using integrated weed management systems to conserve sustainability.

Karim and Bradshaw, 1968, recommended the selection for herbicide- resistance in crop plants. There must be no consideration to loss of fitness with progress of selection for herbicide-resistance, since, crop plants undergone generations of selection.

Research results regarding improvement of alfalfa tolerance to glyphosate in Egypt is relatively scare. The main objective of the recent study was to trace competition indicator of alfalfa populations as affected by recurrent selection to glyphosate tolerance.

MATERIALS AND METHODS

Alfalfa plant materials (*Medicago sativa*, L.*)* used in that recent study will be referred to five base populations. Two cycles of recurrent selection for Glyphosate tolerance were imposed on each of five base populations. (*C.U.F* 101 population Pedigree was (University of California Davis, UC 76, 1972, released by C.U.F seed company). *Siriver* population (Hunter river x *C.U.F* 101 and UC 110 and UC 112),.*Hasawi* population is a land race naturally originated on Saudi Arabia. *Baldi*1 population Selected from EL-Wadi EL-Gedid landrace by Forage Research Department of ARC, Egypt. *Siwa* population is a land race naturally originated on Siwa Oasis of western-desert, Egypt.

Cycle one was practiced on 2800 plants per each base population (C_0). Base populations were seeded at density of 100 $plant.m^{-2}$(considering seed index and germination percentages). Each germplasm seeded in 28 m^2 (20 rows of 1.75m long and 0.80 m apart) on Nubaria Agricultural Research Station, North of Egypt. Seeding date was, May 27th, 2015. Four weeks after seeding, plants (8-15 cm tall) were treated with 0.56 kg acid equivalent per hectare ($ae.ha^{-1}$) of Glyphosate (Round up®) diluted in 480 liter of water (L). Survived plants were left to complete the first cutting growth (two months). Regrowth of the second cutting at 20-25 cm height was sprayed by 0.84 kg ae. ha^{-1} glyphosate in 480 L water. ha^{1}. 14 day after treatment, plants was rated for injury on a 1 to 4 scale (where 1= uninjured, 2=injured shoot, 3=dead shoot with live auxiliary shoots and 4= dead seeding) (Boerboom *et al.*,1991). The uninjured plants were selected uprooted and transplanted to an isolated plots surrounded and covered by insect proof cloth for flowering and seed setting .Plants selected for Glyphosate tolerance from each germplasm were 100 plant .Each germplasm was caged separately in cloth house and a portable honey bees heave (*Apis mellifera, L.)* was used as pollinators (for random matting among plants). Seeds were harvested for each separate plant as a half-sib family on June, 15 th,2016.Equale seed weight from each selected half-sib family seeds were bulked to from first improved cycle (C_1).The second cycle of selection was practiced for each separate improved population. Each population was seeded in 20 rows of 1.25m. long and 0.80 m apart (2000 plant). Four-week-old seedlings were treated with Glyphosate at 0.56 kg ae. ha^{-1} in 480 liters of water. Fourteen days after treatment, injury levels were rated as 1= uninjured, 2=injured shoot, 3=dead shoot with live auxiliary shoots and 4= dead seeding. The uninjured plants were selected uprooted and transplanted to an isolated plot surrounded and covered by insect proof cloth for flowering and seed setting. Plants selected for Glyphosate tolerance from each germplasm were 100 plant. Each germplasm was caged separately in cloth house and a portable honeybees heave (*Apis mellifera L.)* was used as pollinators (for random matting among plants). Seeds were harvested for each separate plant as a half-sib family on June, 15 th, 2017.Equale seed weight from each selected half-sib family seeds were bulked to from second improved cycle (C_2).

Evaluation of selected cycles (C_1 and C_2) along with base populations (C_0) was carried out for each population as a split plot design with Glyphosate treatment (+ and -) as main plots and populations (C_0, C_1 and C_2) as a sub –plot. Four replicates were used. Plot size was three rows of 1.80 m long and 0.15 m apart. Planting of seeds took place at November 1st, 2017. Glyphosate treatment was applied 30 days after planting at 0.84 kg ae. ha^{-1} in 480 liters of water.

Glyphosate treated and untreated plots were evaluated for the following characters:

i- Plant characters: including the following measurements:

1-plant height (cm):as an average of five readings per plot.

2-Number of stems (tilleringpotentiality): counted per a rectangle of 25x25 cm during the successive nine cuttings of evaluation.

ii- Weeds bioassay.

During a three successive cutting of evaluation, weeds were traced in each plot by placing a quadrate of 0.25×0.25 m. Fresh and dry weights of weeds were considered.

Data of all experiments were subjected to analysis of variance according to **Cochran and Cox, 1957**. Means were separated by a protected L.S.D. test (**Fisher, 1960**). **M stat-c** package was used in all analysis.

RESULTS AND DISCUSSION

Plant characters:

Plant characters i.e., plant height (cm) and number of stems /$0.0625m^2$ in all treated and untreated plots by glyphosate, were detected. Table (1) illustrated the mean squares of plant characters (plant height and number of stems) for alfalfa base populations and selection cycles as effected by glyphosate treatment. Significant ($p \geq 0.01$) variations were detected among the glyphosate treatment, alfalfa populations and the interaction between population and selection cycles for plant height and number of stems. While, number of stems had significant variation ($p \geq 0.01$) due to glyphosate x population x selection cycles interaction.

Table (1): Mean squares of plant characters for alfalfa base population and selection cycles affected by glyphosate.

S.O.V	d.f.	M.S	
		Mean of Plant height	Mean of Number of stems
Glyphosate treatment (G)	1	2127**	2547**
Rep /Glyphosate	3	35.90	26.39
Population (P)	4	38.76**	23.08**
GXP	4	9.268*	78.40**
Selection cycles (S)	2	9.836*	27.78**
GXS	2	3.395n.s	1.249n. s
PXS	8	33.29**	19.26**
GXPXS	8	5.002n. s	15.33**
Error	84	2.927	2.515

*, **; significant at 0.05 and 0.01 levels, respectively.
n.s: not significantly different at 0.05 level of probability.

Table (2), presented mean values of plant characters (plant height (cm) and number of stems) as affected by main effect of the studied factors (glyphosate, alfalfa populations and selection cycles). As for, plant height (cm), untreated alfalfa had taller plants than untreated plants (69.48 and 61.06 cm, respectively). Also, population showed variable plant height over glyphosate treatment and selection cycles. Three of the five studied populations expressed similar plant heights (66.29, 66.69 and 65.17cm for *Hasawi*, *Siriver* and Baladi1 populations respectively). While, both of C.U.F 101 and Siwa populations, enjoyed similarly shorter stems (64.69 and 63.52 cm for *C.U.F 101* and *Siwa* populations respectively). The first cycle of selection for glyphosate tolerance was accompanied with significant reduction in plant height (65.77 and 64.78 cm for base population and cycle one of selection, respectively). Meanwhile, further selection cycle (C_2) was associated with a stem length regain to reach that of the base population (65.26 cm for cycle two).

Regarding mean number of stems for glyphosate treatments, alfalfa populations and selection cycles (Table 2), glyphosate treated plots had a smaller number of stems than treated plots (31.28 *vs.* 40.50 stems for treated and untreated plots, respectively). Also, the studied alfalfa populations expressed different number of stems over glyphosate treatments and selection cycles, where, *C.U.F101* and *Siwa* enjoyed the highest significant number of stems (36.89 cmstemfollowed by *Siriver* populations. with number of stems (35.96 and 36.78 stem, respectively). Also, both of *Siriver*and *Hasawi* populations were similar in tillering (35.96 and 35.14 stems, respectively). In the meantime, *Hasawi* and *Baladi* 1 produced significantly similar number of stems per unit area (35.14 and 34.67 plants, respectively). Meanwhile, the first cycle of selection for glyphosate tolerance, although was accompanied with lower number of stems per unit area, that reduction had not reached the level of significance (35.61 and 35.23 stems for C_0 and C_1 respectively). The second cycle of selection for glyphosate tolerance was associated with better tillering that significantly expressed higher number of stems per unit area (36.83 plants).

Table (2): Mean of plant characters for Glyphosate treatments, alfalfa populations and selection cycles.

Factors	Levels of factor	Mean of plant height (cm)	Mean of number of stems
Glyphosate	Treated	61.06	31.28
	Untreated	69.48	40.50
L.S.D $_{0.05}$		2.676	2.295
Population	*C.U.F 101*	64.69	36.89
	Hasawi	66.29	35.14
	Sirivar	66.69	35.96
	Baladi 1	65.17	34.67
	Siwa	63.52	36.78
L.S.D $_{0.05}$		0.977	0.906
Selection cycles	C_0	65.77	35.61
	C_1	64.78	35.23
	C_2	65.26	36.83
L.S.D $_{0.05}$		0.757	0.702

Table (3), presented the effect of the interaction between alfalfa populations and glyphosate treatments. In general, the untreated plants in all studied base populations were of taller plants relative to glyphosate treated populations. Untreated populations of *Hasawi* and *Siriver* enjoyed significantly similar taller plants (71.01 and 70.78 cm, respectively). While, *Siwa* population, significantly showed the shortest stems when treated with glyphosate (58.54 cm). It was valuable to notice that population response to glyphosate treatment in term of reduction in plant height was descending from *Siwa* to *Hasawi* to *Siriver* to C.U.F 101 to *Baladi1* as 14.6, 13.3, 11.5, 10.8 and 10.4% reduction, respectively.

Concerning the effect of glyphosate treatment × population interaction on alfalfa plant tillering expressed as number of stems per unit area **(Table`3)**, untreated plots gave significantly higher number of stems in all studied populations. The least significant tillering was that of *Baladi* 1 population under glyphosate treatment (27.57 stems), while, the highest tillering was expressed by any of *CUF 101*, *Baladi 1* and *Siwa* populations with no glyphosate treatment (41.62, 41.79 and 41.05 for the three populations, respectively). The vulnerability of the studied alfalfa populations to glyphosate treatment in term of reduction in tillering was highest in *Baladi* 1 population that showed 34.03% reduction in tillering ability. While, both of *C.U.F101* and *Hasawi*were of similar vulnerability with reduction in tillering reached 22.71 and 24.54%, respectively. In the meantime, both of *Siriver* and *Siwa* population were the least vulnerable with tillering reduction of 10.73 and 13.45%, respectively.

Table (4) represented the effect of the interaction between alfalfa populations and selection cycle on plant height (cm). In most studied populations, plant height decreased with the first cycle of selection to glyphosate tolerance by -8.991, -3.225, -1.240 and -0.426% in *Siwa*, *Hasawi*, C.U.F101 and *Baladi*1 populations, respectively. Except for, *Sirivar* population an increase by +6.740 % was accompanied with selection to glyphosate tolerance. On the other hand, by cycle two of selection populations had different response relative to C1, where Baladi1, C.U.F101 and *Sirivar* populations had a decrease in plant height with values reached -1.761, -0.720 and 0.617 cm, respectively. While,*Hasawi*and *Siwa* populations showed an increased plant height by +3.816 and +3.197cm, respectively.

Table (3): Mean of plant characters for the interaction between alfalfa populations and glyphosate treatment

Population	Mean of Plant height(cm)		Mean of Number of stems	
	Treated	Untreated	Treated	Untreated
C.U.F 101	60.99	68.39	32.17	41.62
Hasawi	61.57	71.01	30.25	40.04
Sirivar	62.61	70.78	33.93	38.01
Baladi 1	61.60	68.74	27.57	41.79
Siwa	58.54	68.51	32.53	41.05
L.S.D $_{0.05}$	1.382		1.281	

Table (4): Mean of plant characters for alfalfa base population and selection cycles affected by Glyphosate

Population	Selection cycle	Mean of Plant height(cm)	Relative to C_0	Relative to C_1	Mean of Number of stems	Relative to C_0	Relative to C_1
C.U.F 101	C_0	65.39			37.09		
	C_1	64.58	$-1.249^{n.s}$		36.87	$-0.598^{n.s}$	
	C_2	64.11		$-0.720^{n.s}$	36.71		-0.447
Hasawi	C_0	66.91			33.00		
	C_1	64.75	-3.225^{*}		35.76	8.345^{*}	
	C_2	67.22		3.816^{*}	36.68		2.587
Sirivar	C_0	63.96			37.81		
	C_1	68.27	6.740^{*}		33.79	-10.612^{*}	
	C_2	67.85		$-0.617^{n.s}$	36.31		7.433
Baladi 1	C_0	65.74			34.39		
	C_1	65.46	$-0.426^{n.s}$		34.59	$0.521^{n.s}$	
	C_2	64.31		$-1.761^{n.s}$	35.07		$1.452^{n.s}$
Siwa	C_0	66.89			35.79		
	C_1	60.87	-8.991^{*}		35.18	$-1.715^{n.s}$	
	C_2	62.82		3.197^{*}	39.39		11.96^{*}
L.S.D $_{0.05}$		1.693			1.570		

These results match true with the findings recorded by Dekker and Duke 1995. They Said that, the production of glyphosate-resistant crops has been the focus of much research for over a decade. A major problem in the production of glyphosate resistant plants is that its glyphosate is readily translocated to meristems and other metabolic sinks, where, it is concentrated to levels many times that found in leaves. Furthermore, it is not metabolically degraded to a significant extent. So, although the plant may be resistant at the foliar level, the concentrations that accumulate in meristems, flower buds, and other metabolic sinks may overwhelm the resistance mechanism. Johal and Huber (2009), said that, indirect effects of glyphosate on disease predisposition result from immobilization of specific micronutrients involved in disease resistance, reduced growth and vigor of the plant from accumulation of glyphosate in meristematic root, shoot, and reproductive tissues, altered physiological efficiency, or modification of the soil microflora affecting the availability of nutrients involved in physiological disease resistance. Vereecken, (2005) said that, glyphosate can form chelates or complexes with micronutrient metal ions in solution. At physiologically relevant pH levels, and pH levels of most soils, Cu and Zn ions in solution can be relatively strongly complexed with glyphosate, whereas Fe, Ca, Mg, and Mn are complexed to lesser degrees. The ability of glyphosate to complex metal ions, glyphosate has been postulated to affect plant uptake of trace nutrients such as Mn^{2+} or Zn^{2+}. For plants grown in hydroponic solutions, mixed results for glyphosate effects on plants have been shown.

Tillering capacity of alfalfa plants represented by number of stems per unit area as affected by the interaction between population and selection cycle (Table 4) showed that alfalfa population significantly varied in response to selection for glyphosate tolerance, since, the first cycle of selection to tolerance gave reduction in tillering capacity of *C.U.F*101, Siriver and Siwa populations (-0.598, -10.612 and -1.715%, respectively). That reduction was only significant for the first two populations. The progress of selection for glyphosate tolerance (C_2) gave insignificant negative (*C.U.F*101) or positive (*Hasawi* and *Baladi* 1) response. While, expressed significant positive response reached 7.433 and 11.96% relative to tillering potentiality in cycle one of *Siriver* and *Siwa* populations, respectively.

The second order interaction among alfalfa population × selection cycle × glyphosate treatment reflected on tillering capacity as number of stems per unit area was presented in Table 5. Evaluation of selection cycles of all populations under glyphosate treatments, showed significantly lower number of stems relative to the corresponding values under no glyphosate treatment. The magnitude of tillering response to glyphosate treatment varied among populations and selection cycles. In *C.U.F* 101 population, a reduction in tillering was associated with each of first and second cycle of selection, although, had not reached the level of selection (-1.397 and -1.363% relative to each of cycle one and cycle two, respectively). In *Hassawi* population under glyphosate treatment, selection for tolerance was associated with an increase in tillering reached 6.948 and 12.08% relative to C_1 and C_2, respectively. The effect of the second cycle of selection had reached the level of significance. In both of *Siriver* and *Baladi* populations, although tillering under glyphosate treatment was noticed, that change had not reached the level of significance. The soundest change in tillering potentiality was recorded with *Siwa*population, since, a significant reduction of -13.75% relative to (C_0) was associated with the first cycle of selection to glyphosate tolerance. One the other hand, a significant increase of 23.18% relative to cycle two of selection was achieved in plant tillering. It was valuable to notice that the base populations of *Hassawi*, *Baladi 1*cycles and cycle one of *Siwa* presented the least tillering potentiality.

Evaluation of population's cycles under no glyphosate treatment showed that, only *Siriver* and *Siwa* population's cycles had showed significant response to selection for glyphosate tolerance. In *Siriver* population, the first cycle of selection expressed a significant reduction of -16.55% tillering relative to base population. While,the second cycle gave appositive significantresponse of +12.77% relative to first cycle. But in common, selected cycles expressed significantly lower tillering that the base population. In *Siwa* population, the two successive cycles of selection gained higher tillering reached 8.791 and 4.208% relative to each of the preceding cycles, respectively. The tillering of selected cycles was superior to base population.

Commonly, selection for glyphosate tolerance affected the tillering capacity of alfalfa populations with variable magnitudes only in *Siwa* population, the second cycle of selection improved tillering capacity over the first cycle or the base population when evaluated under no glyphosate treatment.

These results match true with the findings recorded by Zobiole *et al*.2011, they suggest that applying glyphosate at early growth stages using the lowest glyphosate rate might have less damage on growth and productivity of RR soybeans.

Weed bioassay:

Glyphosate treated plots (+ and -) were evaluated by weed bioassay in terms of green and dry weight during a course of three successive cuttings. Exposed genetic materials to glyphosate treatments were selection cycles (C0, C1 and C2) of the five studied populations. Mean squares of weeds and dry bioassay for alfalfa populations and selection cycles as affected by glyphosate treatments were presented in Table 6. Glyphosate treatments significantly ($P \geq 0.01$) influenced green bioassay of weeds. Also, the studied populations significantly ($P \geq 0.01$) varied in response to weed invasion and growth determined as green or dry biomass (significant glyphosate × population interaction), along with significant variations among populations to weed invasion and development (green and dry bioassay). The tendency of the studied selection cycles to influence to weed development as a green weight was significantly ($P \geq 0.01$) different. Also, the competition of selected cycles to weeds was significantly influenced by glyphosate treatments (significant glyphosate treatments × selection cycles interaction). Meanwhile, the behavior of selected cycle as a suppressor or simulator to weed development was different ($P \geq 0.01$) in direction or magnitude by the nature of the base population from which it was originated (significant population × selection cycles interaction). Finally, for each glyphosate treatment applied to each of the studied base populations, weed development as a measure of tolerance or intolerance was significantly varied with variable selection cycles. (significant glyphosate treatments × population × selection cycles interaction).

Table (5): Mean of plant characters for interaction between alfalfa base population, selection cycles affected by Glyphosate treated.

Population	Selection cycle	Mean of Plant height(cm)		Mean of Number of stems					
		Treated	Untreated	Treated	Relative to		Untreated	Relative to	
					C_0	C_1		C_0	C_1
C.U.F 101	C_0	61.34	69.44	32.61			41.58		
	C_1	61.33	67.82	32.16	-1.379^{ns}		41.58	0.000^{ns}	
	C_2	60.31	67.90	31.72		-1.363^{ns}	41.69		0.264^{ns}
Hasawi	C_0	62.01	71.80	27.00			39.00		
	C_1	60.02	69.48	30.80	6.948^{*}		40.70	4.358^{ns}	
	C_2	62.69	71.75	32.94		12.08^{ns}	40.41		-0.712^{ns}
Sirivar	C_0	60.25	67.66	34.52			41.08		
	C_1	63.11	73.42	33.30	-3.534^{ns}		34.28	-16.55^{*}	
	C_2	64.46	71.23	33.94		1.921^{ns}	38.66		12.77^{*}
Baladi 1	C_0	61.36	70.12	26.64			42.13		
	C_1	62.31	68.61	28.13	5.593^{ns}		40.99	-2.705^{ns}	
	C_2	61.13	67.49	27.91		-0.782^{*}	42.22		3.000^{ns}
Siwa	C_0	62.91	70.86	33.36			38.22		
	C_1	54.62	67.12	28.77	-13.75^{*}		41.58	8.791^{*}	
	C_2	58.09	67.54	35.44		23.18	43.33		4.208^{ns}
L.S.D $_{0.05}$		n.s		2.227					

n.s.; not significant different.

Table (6): Mean squares of weed green bioassay and weed dry bioassay for alfalfa population and selection cycles as affected by glyphosate treatments.

S.O.V	d.f.	M.S	
		Green bioassy	Dry bioassy
Glyphosate treatment (G)	1	6165**	978.9 n.s.
Rep /Glyphosate	3	370.9	218.6
Population (P)	4	1496**	692.2 **
GXP	4	2737**	1298**
Selection cycles (S)	2	2037**	387.9n.s
GXS	2	1872**	920.9**
PXS	8	2564**	753.7**
GXPXS	8	2017**	441.4**
error	84	174.2	147.8

*,** significance at 0.05 and 0.01 levels of probability, respectively.

n.s.; not significantly different at 0.05 level of probability.

Means of weeds green and dry bioassay (kg. ha^{-1}) as affected by glyphosate treatments and selection cycles were presented in Table (7). Over populations, glyphosate treatment significantly suppressed weed green weight by 13.21%, while, dry weight of weeds was suppressed by 5.275%. The first cycle of selection to glyphosate tolerance expressed a significant increase of 9.59 and 10.93% relative to the base population as green and dry weed weights, when evaluated under glyphosate treating condition. While, the second cycle of selection was associated with a reduction of 29.63 and 28.88% in green and dry weight of weed. This might indicate a proliferation of alfalfa plants that were selected for glyphosate tolerance, the matter the reduced the mass of weeds, whether,as green or dry weight. Evaluation of selected cycles under the absence of glyphosate application significantly expressed variable values of weed bioassay. The first selected cycles of alfalfa showed a reduction of -3.329% in green weight of weeds. Meanwhile, the change in weed biomass as dry weight had not reached the level of significance. The second cycle of selection for glyphosate tolerance was associated with insignificant reduction in weed green weight (-2.64%) and insignificant increase (5.73%) in weeds dry weight. Commonly, the second cycle of selection for glyphosate tolerance significantly showed an obvious suppression to weeds green and dry weights (bioassay) when evaluated under glyphosate treatment.

The relation between selection cycles and alfalfa populations was illustrated in Table 8. C.U.F 101 base population (C_0) was subject to weed invasion. The first cycle of selection (C_1) for glyphosate tolerance significantly reduced the incidence of weeds represented by green biomass by 24.22% relative to what recorded at the base population. Additional selection (C_2) was associated with further reduction in the level of green weed biomass invasion, but, that reduction had not reached the level of significance. *Hasawi* base population (C_0), exhibited the heaviest green weed invasion among all studied base populations. Selection for glyphosate tolerance in *Hasawi* gave substantial reduction in weeds green biomass as 17.04 and 42.55% for cycle one and two, respectively. In *Siriver* population, the first cycle of selection for glyphosate tolerance gave significantly lower alfalfa competition, *i.e*; higher weed green mass invasion at a level reached 22.48% relative to weed green mass at base population. Whereas, the second cycle of selection for glyphosate tolerance suppressed weed green biomass by 13.8% relative to value of cycle one. Similar trend was expressed by *Baladi*1population in response to selection cycles with values amounted to 19.44%increase in weeds green mass after the first cycle of selection to glyphosate tolerance and 21.22% decrease after the second cycle of selection. Paradoxically, selection for glyphosate tolerance is *Siwa* population was associated with significant dimension in alfalfa competition, *i.e*; as increase in weeds green biomass reached 18.05 and 12.14% after cycle one and two relative to each preceding cycle, respectively. Commonly, weeds green biomass response to selection cycles was recorded variably among the studied base populations. A significant reduction in weed green mass was associated with one cycle of selection for glyphosate tolerance in C.U.F 101 and *Hasawi* populations. An opposite responsewere noticed in *Siriver*, *Baladi* and *Siwa*populations. Meanwhile, the second cycle of selection for glyphosate tolerance was associated with a reduction in weeds green biomass in all studied populations, but, *Siwa*. Reduction or increase in weeds green mass is mostly compensated by alfalfa growth and tillering.

Table (7): Means of weeds green and dry bioassay (kg.ha^{-1}) as affected by glyphosate treatments and selection cycles.

Selection cycle	Green bioassyKg.ha^{-1}						Dry bioassyKg.ha^{-1}					
	Treated	Relative to		Untreated	Relative to		Treated	Relative to		Untreated	Relative to	
		C_0	C_1		C_1	C_0		C_0	C_1		C_0	C_1
C_0	156.4			180.2			29.99			31.66		
C_1	171.4	9.590^{*}		174.2	-3.329^{ns}		33.27	10.93^{ns}		32.11	1.421^{ns}	
C_2	120.6		-29.63^{*}	169.6		-2.64^{ns}	23.66		-28.88^{*}	33.95		5.73^{ns}
L.S.D $_{0.05}$	8.263						7.612					

Dry mass of weeds as an indicator for alfalfa vigor and niche occupation showed that selection for glyphosate tolerance in C.U.F 101 population reduced weeds dry mass with progress of selection. That reduction had not reached the level of significance. As for *Hasawi* population, the only significant reduction in dry weeds mass was reached after two cycles of selection (39.71% reduction in weeds dry mass relative to cycle one). Dry mass of weeds failed to indicate the effect of selection for glyphosate tolerance in *Siriver* population. In *Baladi*1, by the second cycle of selection, alfalfa significantly suppressed weeds dry mass by 23.46% relative to cycle one. While dry mass of weeds failed to indicate the influence of selection cycles to vigor and competition of *Siwa* alfalfa.

Table (8): Means of weeds green and dry bioassay (kg. ha^{-1}) as affected by the interaction between alfalfa populations and selection cycle

Population	Selection cycle	Green Bioassay kg.ha^{-1}			Dry Bioassay kg.ha^{-1}		
		Means	Relative to		Means	Relative to	
			C_0	C_1		C_0	C_1
C.U.F 101	C_0	92.79			47.44		
	C_1	70.31	-24.22*		37.42	-21.72^{ns}	
	C_2	66.80		-4.992^{ns}	40.84		9.139^{ns}
Hasawi	C_0	112.8			62.33		
	C_1	93.57	-17.04*		54.44	-12.65^{ns}	
	C_2	53.75		-42.55*	32.82		-39.71*
Siriver	C_0	71.83			42.14		
	C_1	93.01	22.48*		51.52	22.25^{ns}	
	C_2	80.84		-13.08^{ns}	45.33		-12.01^{ns}
Baladi 1	C_0	90.45			48.02		
	C_1	108.04	19.44*		61.79	28.67*	
	C_2	85.11		-21.22*	47.29		-23.46*
Siwa	C_0	81.05			46.73		
	C_1	95.68	18.05*		56.44	20.77^{ns}	
	C_2	107.3		12.14^{ns}	64.19		13.73^{ns}
L.S.D $_{0.05}$		13.06			12.03		

Evaluation of weed biomass influence to selected cycles of the studied alfalfa populations evaluated under glyphosate treatments (+ and – were expressed in Table 9. Green mass of weeds in base populations of *C.U.F* 101, *Siriver* and *Baladi*1were not affected by glyphosate treatments. While, green weed mass was suppressed by glyphosate treatment in *Hasawi* and *Siwa* populations. The first cycle of selection for glyphosate tolerance in *C.U.F* 101 population, resulted in a competitive alfalfa plants (reduced green mass of weeds), whether, evaluated as glyphosate positive or negative treated that effect was only noticed in *Hasawi*population under glyphosate untreated plots. Oppositely, less competitive alfalfa plants to weeds green mass were recorded in *Baladi*1and *Siwa* populations under glyphosate treatment. Also, similar response was noted in *Siriver*population when evaluated in absence of glyphosate treatment. Meanwhile, insignificant influences of cycle one selection to alfalfa competition were expressed in *Hasawi* and *Siriver* population under glyphosate treatment and *Baladi*1and *Siwa* populations under no glyphosate treatment.

While, green bioassay of weeds partially explained the influence of selection to glyphosate tolerance to competitiveness and vigor of produced selection cycles, dry bioassay of weeds failed to indicate any significant influence of selection cycles and glyphosate treatments to competitiveness of alfalfa populations.

Table (9): Means of weeds green and dry bioassay (kg.ha^{-1}) as affected by the interaction between alfalfa populations, Selection cycle and glyphosate treatments

Population	Selection cycle	Green bioassay (kg.ha^{-1}) Glyphosad treated	Relative to C_0	Relative to C_1	Glyphosad untreated	Relative to C_0	Relative to C_1	Dry bioassay (kg.ha^{-1}) Glyphosad treated	Relative to C_0	Relative to C_1	Glyphosad untreated	Relative to C_0	Relative to C_1
C.U.F 101	C_0	180.2			167.7			32.07			27.23		
	C_1	131.4	-27.08*		132.2	-21.16*		25.27	-21.20^{ns}		21.51	-21.00^{ns}	
	C_2	103.2		-21.46*	147.2		11.34^{ns}	19.29		-23.66	31.76		47.65^{ns}
Hasawi	C_0	181.2			241.9			37.36			40.56		
	C_1	198.1	9.326^{ns}		152.8	-36.83*		41.53	11.16^{ns}		26.52	-34.61^{ns}	
	C_2	123.1		-37.85*	78.46		-48.65*	25.68		-38.16	15.34		-42.15^{ns}
Siriver	C_0	131.0			138.3			23.70			28.97		
	C_1	124.7	-4.809^{ns}		224.0	61.96*		23.71	0.042^{ns}		40.59	40.11^{ns}	
	C_2	72.62		-41.71*	230.5		2.90^{ns}	13.60		-42.64	43.06		6.085^{ns}
Baladi 1	C_0	148.3			190.8			28.19			31.84		
	C_1	214.9	44.90*		190.2	-0.314^{ns}		39.65	40.65^{ns}		37.58	18.02^{ns}	
	C_2	168.4		-21.63*	150.7		-20.76*	31.03		-21.7	28.08		-25.27^{ns}
Siwa	C_0	141.6			162.2			28.63			29.72		
	C_1	187.7	32.55*		171.6	5.795^{ns}		36.21	26.41		34.34	15.54^{ns}	
	C_2	136.0		-27.54*	241.3		40.61*	28.72		-20.68^{ns}	51.52		50.02*
L.S.D $_{0.05}$		18.47						17.02					

The recent results might be explained depending on the competitive relations among alfalfa plants and invading weeds. Also, reduction in plant density following glyphosate application encourage the proliferation of weeds in the niche of field. Shaner (2000), recommended the use of glyphosate as a selective, post-emergence weed control measure in glyphosate- tolerant soybean, cotton, canola and maize. Reddy (2001) stated that evaluation of entries under glyphosate treatment, determine early season weed interference to crop development. Dill *et al* (2008) supported last finding through recommending glyphosate- ready crops as a measure to eliminate and minimize weed invasion. Green (2012) reported that, although, glyphosate had used extremely well in controlling weeds in glyphosate- resistant crops for long times, some key weeds had evolved resistance and dominated in fields. Miller *et al* (2006) stated that glyphosate tolerant crops, requires application of specific herbicides to control tolerant weeds. An explanation to dispersion of weeds in a glyphosate resistant or tolerant crop field Busi and Powles (2009) stated that, selection of glyphosate tolerant crop by plants exposure to sub- lethal dose of glyphosate, includes the contribution of minor genes endowing substantial plant survival at sub lethal herbicide dose might be potential complementary path to herbicide resistance evolution in weed populations. Also, Zenk (1974) reached that, selection pressure that might be imposed in the field can rarely be made to exceed 90-95% kill, while, majority of remaining plants are "escapees" Also, high dose rate in field application, hardly increases kill of susceptible plants, but, may be lethal to the few truly resistant plants among the escapees.

CONCLUSION

The major obtained results might be summarized in the following:

Plant height (cm):

– In most studied populations, plant height decreased with the first cycle of selection to glyphosate tolerance by -8.991, -3.225, -1.240 and -0.426% in *Siwa*, *Hasawi*, C.U.F101 and *Baladi*1 populations, respectively. Except for, *Sirivar* population an increase by +6.740 % was accompanied with selection to glyphosate tolerance.

– On the other hand, by cycle two of selection, populations had different response relative to C1, where Baladi1, C.U.F101 and *Sirivar* populations had a decrease in plant height with values reached -1.761, -0.720 and 0.617 cm, respectively. While, *Hasawi*and *Siwa* populations showed an increased plant height by +3.816 and +3.197cm, respectively.

Number of stems:

– The first cycle of selection for glyphosate tolerance, although was accompanied with lower number of stems per unit area, that reduction had not reached the level of significance (35.61 and 35.23 stems for C_0 and C_1 respectively).

– The second cycle of selection for glyphosate tolerance was associated with better tillering that significantly expressed higher number of stems per unit area (36.83 plants).

– Alfalfa population significantly varied in response to selection for glyphosate tolerance, since, the first cycle of selection to tolerance gave reduction in tillering capacity of C.U.F101, *Siriver* and *Siwa*populations (-0.598, -10.612 and -1.715%, respectively). That reduction was only significant for the first two populations.

– The progress of selection for glyphosate tolerance (C_2) gave insignificant negative (C.U.F101) or positive (Hasawi and Baladi 1) response. While, expressed significant positive response reached 7.433 and 11.96% relative to tillering potentiality in cycle one of Siriver and Siwa populations, respectively.

Weed Bioassay:

– Glyphosate treated plots (+ and -) were evaluated by weed bioassay in terms of green and dry weight during a course of three successive cuttings. Exposed genetic materials to glyphosate treatments were selection cycles (C_0, C_1 and C_2) of the five studied populations.

– The first cycle of selection to glyphosate tolerance expressed a significant increase of 9.59 and 10.93% relative to the base population as green and dry weed weights, when evaluated under glyphosate treating condition.

– The second cycle of selection was associated with a reduction of 29.63 and 28.88% in green and dry weight of weed. This might indicate a proliferation of alfalfa plants that were selected for glyphosate tolerance, the matter the reduced the mass of weeds, whether,as green or dry weight

– Commonly, the second cycle of selection for glyphosate tolerance significantly showed an obvious suppression to weeds green and dry weights (bioassay) when evaluated under glyphosate treatment.

– The relation between selection cycles and alfalfa populations might be discussed as follows.

- C.U.F 101 base populations (C_0) was subject to weed invasion. The first cycle of selection (C_1) for glyphosat tolerance significantly reduced the incidence of weeds represented by green biomass by 24.22% relative to what recorded at the base population.

- Additional selection (C_2) was associated with further reduction in the level of green weed biomass invasion, but, that reduction had not reached the level of significance.
- *Hasawi* base population (C_0), exhibited the heaviest green weed invasion among all studied base populations. Selection for glyphosate tolerance in *Hasawi* gave substantial reduction in weeds green biomass as 17.04 and 42.55% for cycle one and two, respectively.
- In *Siriver*population, the first cycle of selection for glyphosate tolerance gave significantly lower alfalfa competition, *i.e*; higher weed green mass invasion at a level reached 22.48% relative to weed green mass at base population. Whereas, the second cycle of selection for glyphosate tolerance suppressed weed green biomass by 13.8% relative to value of cycle one.
- Similar trend was expressed by *Baladi*1population in response to selection cycles with values amounted to 19.44% increase in weeds green mass after the first cycle of selection to glyphosate tolerance and 21.22% decrease after the second cycle of selection.
- Paradoxically, selection for glyphosate tolerance is *Siwa* population was associated with significant dimension in alfalfa competition, *i.e.*, as increase in weeds green biomass reached 18.05 and 12.14% after cycle one and two relative to each preceding cycle, respectively.

– While, green bioassay of weeds partially explained the influence of selection to glyphosate tolerance to competitiveness and vigor of produced selection cycles, dry bioassay of weeds failed to indicate any significant influence of selection cycles and glyphosate treatments to compete-tiveness of alfalfa populations.

LITERATURE CITED

Boerboom C.M, N.J, Ehlke D.L Wyse and D.A Somers. 1991. Recurrent selection for glyphosate tolerance in Birdsfoot Trefoil. Crop sci. 31;1124-1129.

Busi, R. and S.B. Powles. 2009.Evalution of glyphosate resistance in a *Loliumrigidum* population by glyphosate selection at sublethal doses. Heredity 103; 318-325.

Cochran , W.G. and Cox,G.M.1957. Experimental Design.2nd .John Wiley, N.Y, U.S.A.

Dekker.J. and S. O. Duke 1995. Herbicide-Resistant Field Crops. Iowa State University. http://lib.dr.iastate.edu/agron_pubs .

Dill G.M,C.A. CaJacob and S.R. Padgette 2008. Glyphosate-resistant crops: adoption, use and future considerations. *Pest Manag Sci* 64: 326–331.

Fisher ,R.A. 1960. The Designs of Experiments. 7 th. Ed. Oliver&Boyd, Edinburgh.

Gottrup,O., P.AO, Sullivan, R.J.Schraa and W.H. Vanden Born. 1976. Uptake, translocation, metabolism and selectivity of glyphosate in Canada thistle and leafy spurge. Weed Res. 16-197:201.

Green.J.M.2012.The benefits of herbicide-resistant crops. *Pest Manag. Sci*. 68; 1323-1331.

Johal G.S.and D.M. Huber 2009. Glyphosate effects on diseases of plants. Europ. J. Agronomy 31; 144-152.

Karim, A., and A. D. Bradshaw. 1968. Genetic variation in simazine resistance in wheat, rape and mustard. Weed Res., 8; 283-29.

Miller S.D, R.G, Wilson A.R Kniss and C.M.Alford 2006.Roundup Ready® Alfalfa A new technology for high plains hay producers. Jenna Norfolk, College of Agriculture intern.

MSTAT-C.(1996). Russel, D.Freed, MSTAT Director, Crop and soil Sciences Department, Michigan State University , U.S.A.

Neal, J. C, W. A. Skrock and T.J.Monaco.1985. Effect of plant growth stage on glyphosate. Plant phsiol.,76:551-574.

Orloff. S. and Putnam .D.2011.Roundup ready® alfalfa- what have we learned to date.Western Alfalfa & Forage Conference,

http://alfalfa.ucdavis.edu.

Reddy K. N, 2001. Glyphosate-resistant soybean as a weed management tool: Opportunities and challenges. Weed Biology and Management 1;193:202.

Shah, D.M., R.B Horsch, H .J. Klee, G.M, Kishore J .A. Winter, N. E. Turner, C.M. Hironaka, P .R. Sanders, C.S .Gasser, S .Aykent, N .Siegel, S .G Rogers and R .T. Fraley,1986 . Engineering herbicide tolerance in transgenic plants .Science 233 : 478-481.

Shaner, D.L.2000.The impact of glyphosate-tolerant crops on the use of other herbicides and on resistance management. Pest Manage Sci. 56,320-326.

Zenk, M. H. 1974. Haploids in physiological and biochemical research, in: "Haploids in Higher Plants: Advances and Potentials," K. J. Kasha, ed., Univ. of Guelph, Guelph, Canada.

Zobiole L. H. S, R.J Kremer, R. S. Oliveira and .J Constantin. 2011. Glyphosate affects chlorophyll, nodulation and nutrient accumulation of ‘‘second generation’’ glyph-osate-resistant soybean (*Glycine max*, L.) a Pesticide Biochemistry and Physiology 99; 53-60.

دلائل القدره على المنافسه فى عشائر البرسيم الحجازى بعد الانتخاب الدورى لتحمل الجليفوسات

محمد عبد الستار احمد و احلام حسنى مصطفى و سناء ابراهيم ميلاد و طه احمد محمود

الجانب السئ في تبني صنف البرسيم الحجازي المتحمل لمبيد الحشائش هو التغير الحادث في عشائر الحشائش مع تغلب نسبي للأنواع المتحملة. الهدف الرئيسي للدراسة الحالية هو تتبع دلائل القدرة علي المنافسة في عشائر البرسيم الحجازي بعد تأثرها بالانتخاب الدوري لتحمل الجليفوسات. تم إجراء دورتان من الانتخاب الدوري لتحمل الجليفوسات لكل عشيرة من عشائر البرسيم الحجازي. وقد اعتمد علي صفات النبات والتقييم الحيوي للحشائش كدلائل للقدرة التنافسية للبرسيم الحجازي. في معظم العشائر المدروسة تناقص إرتفاع النبات بعد الدورة الأولي للانتخاب من تحمل الجليفوسات بحوالي −٨,٩٩١ و ٣,٢٢٥ و −١,٢٤٠ و −٠,٤٢٦ وذلك في عشائر سيوة وحساوي و C.U.F. 101 وبلدي١ علي الترتيب. وفيما عدا العشيرة ساي ريفر التي أظهرت زيادة في إرتفاع النبات مقدارها +٦,٧٤% مع الانتخاب لتحمل الجليفوسات. ومن ناحية آخري فمع الدورة الثانية من الانتخاب سجلت العشائر استجابة مختلفة منسوبة إلي الدورة الأولي حيث سجلت العشائر بلدي١ و C.U.F. 101 وساي ريفر نقص في إرتفاع النبات بقيم بلغت −١,٧٦١ و −٠,٧٢٠ و ٠,٦١٧سم علي الترتيب. بينما أظهرت عشيرة حساوي وسيوة زيادة أرتفاع النبات بمقدار ٣,٨١٦ و ٣,١٩٧ سم علي الترتيب. وقد تم تقييم القطع التجريبية المعاملة والغير المعاملة بالجليفوسات من حيث التقييم الحيوي للحشائش معبراً عنه بالوزن الأخضر والجاف خلال ثلاث حشات متتالية , حيث تم معاملة ناتج دورات الانتخاب الأولي والثانية وعشيرة الاساس للعشائر المدروسة الخمسة بمبيد الجليفوسات. وقد أظهرت الدورة الأولي من الانتخاب لتحمل الجليفوسات زيادة بمقدار ٩,٥٩ و ١٠,٩٣% منسوباً إلي عشير ة الاساس في كلاً من الوزن الأخضر والوزن الجاف عند تقيمهما تحت ظروف الرش بالجليفوسات أما الدورة الثانية من الإنتخاب, فقد ارتبطت بنقص مقداره ٢٩,٦٣ و ٢٨,٨٨% في كلاً من الوزن الأخضر والجاف في الحشائش وقد يعبر هذا عن زيادة في القدرة التنافسية لنباتات البرسيم الحجازي التي تم إنتخابها لتحمل مبيد الجليفوسات.