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Germination performance of some legume crops under varying filter paper mostures

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Abstract

Adverse effects of water stress without ions interactions in Baraka, Adlib and Nineveh lentil cultivars have been investigated. Results showed that germination under saturation (o AWC depletion %) was the paramount treatment as it manifested the highest values. It profoundly surpassed that of 25, 50, and 75 AWC depletion % in terms of final germination percentage (3.9, 7.3, and 22.9), germination rate (51, 66.2 and 97.7%), radical length (65.9, 127.5 and 204.9%), plumule length (51.7, 127.5 and 430.6%), respectively. Additionally, this treatment highly reduced the days required for peak germination. Regression analysis revealed that final germination percentage was linearly responded to different moisture and can be forecasted by the following equation: Y= 100-0.234X where, (r2=36.6). Mungbeans was the most potent cultivars, which significantly surpassed Nineveh, Adlib, Baraka and Common Vetch in final germination (3.3, 2.6, 4.3 and 17.8%), plumule length (102.7, 82.5, 119.6 and 96.4%), respectively. It also unveiled superiority in shortening the time required for peak germination, days required for first emergence and radical plumule lengths, in compression to other cultivars. These results suggested that the superiority order should be as follow: Mungbean > Adlib > Nineveh > Baraka >Common Vetch. Adlib seed germinated on filter paper saturated by distilled treatment shoed preponderance other interaction treatments in radical length (121.25 mm). Moreover under severe water deprivation (depletion of 75% AWC), Adlib dominance was also unveiled as it gave the highest radical length (51.25 mm), final germination percentage (90%), respectively.

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Introduction

Seed germination, beginning with imbibitions and culminating in radical emergence, is an essential process in plant development. Successful stand establishment, in terms of the rapidity and extent of establishment, are contingent on adequate seed germination and vigor. Moreover, the rapidity and extent of stand establishment are important factors determining yield and maturity of spring crops (Briggs and Aytenfisu, 1979). Water uptake by the seed generally occurs in three phases: rapid initial uptake, a lag phase with limited further uptake, then a third phase of rapid water uptake associated with radical emergence (Bewley and Black, 1994). The process of imbibitions has been clearly described in maize (McDonald et al., 1994). They suggested that water is taken up through two separate pathways, the first resulting in near full embryo hydration within 15 h, and the second resulting in endosperm hydration that can take longer than 48 h. At germination, the moisture content of the embryo was > 50%, whereas the moisture content of the endosperm was still 25-30%, and it subsequently continued to wet up.

In maize the embryo is positioned to one side of the seed and imbibitions of the embryo is more rapid if that side, rather than the side containing only endosperm, is in contact with moisture. Maize seeds were always placed embryo-side down in contact with the membrane. In order to reduce any additional variation in imbibitions between individuals, all chickpea seeds were also placed in the same orientation with the embryonic axis in contact with the membrane (Finch-Savage et al., 2005). The longer the germination period of leguminous seeds are the greater the reduction of α -galactosides contents. The same conclusion, formulated by the sentence: "There is a positive correlation between the decline in agalactosides content and the depression of seed longevity", is shown in the book by Gorecki et al. (1997). The legume seeds contain albumin and globulin storage proteins; act as amino acid reserves which are

mobilized to nourish the seedling. Globulins belong to the vicillin and legumin family these are degraded by endoproteases particularly cysteine proteinase. In horse gram, during germination we observed the disappearance of high molecular weight (HMW) polypeptides of globulins and appearance of a new 25 kDa polypeptide (Zakharov et al., 2004). It appears that the newly synthesized protease is inactive, and activation takes place during germination. The reported enzyme has broad substrate specificity and stability in pH, temperature, etc., therefore, this protease may turn out to be an efficient choice in pharmaceutical, medicinal, food, and biotechnology industry (Jinka et al., 2009). Water potentials were selected to span the range around and below the mean base water potential where maize and chickpea seeds were likely to become metabolically active. It is well understood from osmotic priming studies that seeds exposed to such water potentials will germinate more quickly on removal of the stress than seeds that have not received the treatment. This advancement is generally greater as the degree of stress is reduced and treatment time is increased, but experience has shown little increase beyond 10 d (Rowse et al., 2001). Kaufmann and Ross (1970) had recommended the use of filter paper wetted with PEG; he obtained similar germination percentage with that of soil. Polyethylene glycol 20000 Mw highly affected the capacity of seed germination, particularly at higher concentration rate (Abdel and Salih, 1994). They also found that high germination percentages were observe after PEG stresses removal by rinsing the treated seeds with distilled water which explained the nontoxic effects of PEG, since 81% of the germinated seeds were achieved after rinsing PEG.

Subjecting seeds of varying crops to water stress environments are well established through germinating these seeds in osmotic media including salts ions namely NaCl, KNO₃ KH₂PO₄ and micronutrients. These osmotic solutions reveal ion efflux to the cytoplasm of cells causes cytoplasmic potential reductions and even enzymes denaturations. Some studies mannitol was utilized to create water stress environment as it is a compatible solutes, where no damage might be occurred. Recently, studies were highlightened on poly ethylene glycol (PEG) of high molecular weights to insure no permeation to cytoplasm of cells. However, PEG media suffer anoxia and thus required aerations (Alvarado and Bradford, 2002; Rowse and Finch-Savage, 2003). They reported that humidified enriched air (75% oxygen and 25% nitrogen) diffused through sintered plate gas diffusers in to 8000 PEG at 0.6 liters per minute resulted in greater rate, synchrony, and percentage germination of onion seeds than normal aeration and thus enriched air is viewed as a way of aerating PEG for large scale priming. Therefore, for more safety filter papers wetted to varying extent were adopted to investigate the influence of water stress alone on germination of some legume seeds. The objective of this experiment was to evaluate the adverse effects of water availabilities on germination performance of three lentil cultivars besides common Vetch and mungbeans without ion interferences.

Materials and methods

A laboratory attempt was made to evaluate germination performances of three lentil cultivars namely Nineveh, Adlib and Baraka, besides Common Vetch and Mungbean under varying osmotic potential solutions. This investigation was fulfilled at the laboratory of Field Crops Department, College of Agriculture, Salahalddin University, Erbil, Kurdistan Region, Iraq. Factorial Randomized Complete Block Design was used in this experiment where factor (A) contained four water availabilities levels. Distilled water saturated filter paper 100% water available capacity AWC (a1), 25% AWC depletion (a2), 50% AWC depletion $(a_3, 75\%$ AWC depletion (a_4) . Whereas factor (B) was represented by Nineveh lentil cv. (b₁), Adlib lentil cv. (b₂), Baraka lentil cv. (b₃), Local Common Vetch cv. (b_4) and Local Mungbean cv. (b_5) . Subsequently, 20 treatments were included in this

investigation. Every treatment was replicated 4 times and one replicate contained 4 plastic disposable dishes with their given filter combinations and used to germinate 25 seeds. Four watmann filter papers were wetted with distilled water, four wetted filter paper plus 1 dry filter paper, four wetted filter paper plus 2 dry papers, and four wetted filter plus three dry papers were sealed and left for 2 days used to distribute moisture uniformly in all included filter paper. Therefore, these varying moistened levels of filter papers were considered corresponding to 100% AWC, 25% AWC depletion, 50% AWC depletion, and 75% AWC, respectively. Duration required for peak germination (days), and days for emergence commencements were counted. Final germination percentage, germination energy percentage were calculated from dividing number of germinated seeds on total seeds and from yield of number of germinated seeds after three days from starting divided on the total seeds, respectively, (Ruan et al., 2002).

Germination rate: germination percentage ratio was calculated from dividing the Germination rate over germination percentage. Radical and plumule lengths (mm) were measured by mini roller. While radical Plumule Length ratio was calculated by dividing the length of radical over the length of plumule. Germination rate (seedling.d⁻¹) was calculated from the following formula (Carleton, 1968): SG = No. of grains emerged at first count / Days of first count + ...+ No. of grains emerged at final count / days of final count. Mean germination time (days) was calculated from the equation below:

$$(MGR = \frac{\Sigma nidi}{N})$$

Where ni= number of germinated seeds on day I, d= rank order of day i (number of days counted from the beginning of germinated), and N=total number of germinated seeds. Finally, data were analyzed by computer statistical program me, using Duncan's Multiple Range Test at α = 0.05 probability level.

Results and discussion

Germination performance in response to matric potentials

The obtained results showed that germination under saturation (o AWC depletion %) was the paramount treatment as it manifested the highest values. It profoundly surpassed that of 25, 50, and 75 AWC depletion % in terms of final germination percentage (3.9, 7.3, and 22.9%, respectively), germination energy (13.6, 35.3, and 74%, respectively), germination rate (51, 66.2 and 97.7%, respectively), germination rate: germination percentage ratio (45.7, 55.7 and 55.7% respectively), radical length (65.9, 127.5 and 204.9% respectively), plumule length (51.7, 127.5 and 430.6%, respectively). Additionally, this treatment highly reduces the days required for peak germination (48.6, 75.7 and 84.3%, respectively) days for first emergence (11.1, 58.3 and 75%, respectively) and radical: plumule lengths ratio (8.8, 12.6 and 127.1%, respectively) (Table, 1; Figure, 1-5). Depletion of 25% of AWC% can be considered as the second treatment after saturation. Depletion of 50% of AWC% can be considered as the second treatment after saturation. Therefore, this treatment hugely exceeded that of 75% AWC depletion in terms of final germination percentage (14.5%), germination energy (28.7%), germination rate (18.9%), radical length (33%), and plumule length (133.1%) and with 75% depletion only in radical: plumule lengths ratio (12.5 %). Furthermore this treatment highly reduces the days required for peak germination (4.9%) days for first emergence (10.5%) and radical: plumule lengths ratio (101.7%). This treatment showed superiority over 0, 25 and 75% AWC depletion in mean germination time (27.1, 55.2 and 16.3%, respectively). The lowest values were accompanied by 75% depletion of AWC accept that of days required for peak germination (6.45), days required for first emergence (3.15) and radical: plumule length ratio (4.4). Regression results analysis manifested that all detected traits were linearly correlated to varying moisture availabilities and may be forecasted by the equations (Table, 2), These results

are with accordance to those reported by Abdel and Adnan Salih (1994). They stated that the ratios of wet: dry filter paper layers were applied to germinate fenugreek seeds under varying water availabilities. Low water availabilities substantially reduced seed germination capacity, especially at that of 2:6 ratios which resulted in apparent increases in time required for first emergence, time required for peak germination and germination rate, as compared to these of 2:0 ratio. However, it showed reductions in peak germination percentage and final germination percentage, respectively, as compared to 2:0 ratios. Rewetting filter paper with distilled water to release water stress effects resulted in profoundly high germination percentage. Yet the water stress influences can be raveled by utilizing filter paper where no ions intermingling. The low water availabilities have a direct effect on energy producing, hydrolytic enzymes and reserve translocation from endosperms and/or cotyledons to its destination at the embryo axis. During germination, the seeds (e.g. beans, peas, lentils) are transformed from the dormant state into a metabolically active state. This process involves intensive mobilization of the stored reserves, which results in a rapid increase in respiration, the synthesis of proteins and nucleic acids, and the elongation and division of cells (Gorecki et al., 1997). Glucose, fructose, galactose, sucrose, and α -galactosides (raffinose, verbascose, and stachyose) were found in the samples of all three seed legume types.

Cultivar responses

Results (Table, 1; Figure. 1-5) showed that Mungbean was the most potent cultivars. It significantly surpassed Nineveh, Adlib, Baraka and Common Vetch in final germination (3.3, 2.6, 4.3 and 17.8%, respectively), germination energy (21.1, 14.4, 39.6 and 118.3%, respectively), and germination rate: germination percentage ratio (54, 52.4, 62%, and 59.8%, respectively) and plumule length (102.7, 82.5, 119.6 and 96.4%, respectively). It also unveiled

superiority in shortening the time required for peak germination (165.8, 150, 155.2 and 150%, respectively), days required for first emergence (84.3, 78, 73.9 and

117.3%, respectively)and radical: plumule lengths(54.2, 68.6, 101.4and 8.7%, respectively), incompressiontoothercultivarsaswell.

Treatme		Final Germ. (%)	Mean Germ. Time (days)	Germ. Energy (%)	Germ. Rate (seedling.day)	Days for Peak Germ.
AWC depletion (%)	Control	99.25a	1.67b	94.0a	56.4a	3.5d
	25	95.5b	1.367c	82.75b	37.35b	5.2e
	50	92.5c	2.122a	69.5c	33.925c	6.15b
	75	80.75d	1.825b	54.0d	28.515d	6.45a
Leg	Ν	93.75b	1.984ab	80.0c	36.30b	6.313a
Legume Crops	А	94.375b	1.997a	84.688b	37.091b	5.938b
Cro	В	92.813b	1.806b	69.375d	34.2c	6.063ab
ps	Common Vetch	82.188c	1.309c	44.375e	29.538d	5.938b
	Mungbean	96.875a	1.724b	96.875a	58.181a	2.375c
Cor	Ν	97.5ab	1.938cf	93.75ac	47.425cd	4.75d
Control	А	100a	1.95cf	97.5ab	84.925c	3.5fg
	В	98.75ab	2.025be	95ac	47.05d	4.25de
	Common Vetch	100a	1.438gi	83.75de	38.6e	4.0ef
	Mungbean	100a	1.0jk	100a	100a	1.0i
259 Def	Ν	95bc	1.125ik	90bd	35.1f	6.0c
25% AWC Depletion	А	95bc	1.175ik	90bd	36.025f	6.0c
)n VC	В	95bc	1.375gj	81.25ef	32.075gh	6.0c
	CommonVetch	92.5cd	1.275hj	52.5j	29.8i	6.oc
	Mungbean	100a	1.885df	100a	53.75b	2.0h
50% Del	Ν	92.5cd	2.388ab	75.0fg	32.8g	7.0ab
50% AWC Depletion	А	92.5cd	2.588a	82.5de	32.7g	7.0ab
on VC	В	92.5cd	2.25ad	65.0hi	30.425hi	7.0ab
	Common etch	85f	1.688eg	25.0l	25.975j	6.75b
	Mungbean	100a	1.698eg	100a	47.725cd	3.0g
75% AWC Depletion	Ν	90de	2.125bd	61.25i	29.875i	7.5a
	А	90de	2.275ad	68.75gh	30.425hi	7.25ab
йÖ	В	85f	1.575fh	36.25k	27.25j	7.0ab
	Common Vetch	51.25g	0.838k	16.25m	23.775k	7.0ab
	Mungbean	87.5ef	2.313ac	87.5ce	31.25gi	3.5fg

Table 1a. Germination and seedling performances in different cultivars.

Treatments		Days for	Germ. Rate:	Radical	Plumule	Radical Plumule
		First	Germination	Length	Length	Length ratio
		Emerg.	Percentage	(mm)	(mm)	0
		-	Ratio			
de	Control	1.8d	0.567a	109.95a	62.35a	1.939c
AWC depletion (%)	25	2.0c	0.389b	66.25b	41.1b	2.109bc
AWC (%)	50	2.85b	0.364c	47.95c	27.4c	2.183b
on	75	3.15a	0.364c	36.05d	11.75d	4.404a
	Ν	2.65b	0.385b	71.563b	29.25c	2.797c
o Fe	А	2.563b	0.389b	80.0a	32.5b	3.058b
Legume Crops	В	2.5b	0.366c	71.25b	27.0d	3.653a
)s	Common Vetch	3.125a	0.371c	57.813c	30.188c	1.972d
	Mungbean	1.438c	0.593a	44.625d	59.313a	1.814d
	Ν	2.0d	0.486c	117.5ab	52.5d	2.265de
Co	А	2.0d	0.489c	121.25a	57.5c	2.11df
Control	В	2.0d	0.476c	113.75bc	51.25d	2.228de
rol	Common Vetch	2.0d	0.384d	88.75d	42.5e	2.095df
	Mungbean	1.0e	1.0a	108.5c	108a	0.996h
0.12	Ν	2.0d	0.369de	73.75e	31.25g	2.363de
5% lep	А	2.0d	0.377d	85d	36.25f	2.378de
olet	В	2.0d	0.338eh	72.5e	27.5gh	2.55d
25% AWC depletion	Common Vetch	3.0c	0.322gi	56.25g	36.25f	1.578g
р <u>О</u>	Mungbean	1.0e	0.538b	43.75ij	74.25b	1.699fg
50% AWC depletion	Ν	3.0c	0.355df	48.75hi	22.75i	2.143df
	А	3.0c	0.354dg	62.5f	26.5hi	2.363de
	В	3.0c	0.329fi	62.5f	24.25hi	2.585d
	Common Vetch	3.5b	0.305i	46.25hi	25hi	1.85eg
	Mungbean	1.75d	0.477c	19.75l	38.5f	1.973eg
75% AWC depletion	Ň	3.5b	0.332fi	46.25hi	10.5k	4.418c
	А	3.25bc	0.338eh	51.25g	9.75k	5.4b
	В	3.0c	0.32hi	36.25k	5.0l	7.25a
IOL	Common Vetch	4.0a	0.471c	40jk	17j	2.365de
р С	Mungbean	2.0d	0.357df	6.5m	16.5j	2.588d

Table 1b. Germination and physiological features in different cultivars.

Adlib lentil cultivar come next in superiority this cultivar exceeded Nineveh, Baraka, Common Vetch and Mungbean, in radical length (11.7, 12.2, 38.3 and 79.2%, respectively). While in the time of germination mean, Adlib was superior

over Baraka, Common Vetch and Mungbean by 10.6, 52.6, and 15.8%, respectively. Adlib cultivar was the best in germination energy, germination rate, as compared to Nineveh, Baraka, and Common Vetch in germination energy, germination rate and plumule length by (5.8 2.2, 7.6% respectively), (22, 6.1 and 20.4%, respectively) and (90.8, 25.5 and 7.7%, respectively). It also exceeded Baraka and Common Vetch in germination rate: germination ratio by (6.3 and 4.9%, respectively). Adlib substantially reduced days for peak germinations (6.3%) as compared to Nineveh, and days for first emergence in relation Common Vetch (21.9%). Nineveh cultivar was categorized in the third order it significantly exceeded Baraka cultivar in germination energy (15.3%), germination rate (6.1%) and plumule length (8.3%). The fourth cultivar in superiority was Baraka, as it was exceeded Common Vetch in final germination percentage (12.9%), mean germination time (38%), germination energy (56.3%), germination rate (15.8%) and radical length (23.2%). Therefore, the worst cultivar was Common Vetch as it manifested the lowest values in term of final germination percentage germination time (1.3 (82.2%), mean day), germination energy (44.4%), and germination rate (59.5 seedling.d⁻¹). These results suggested that the superiority order should be as follow: Mungbean> Adlib> Nineveh> Baraka>Common Vetch. Pill (1995) reported that to precisely understand cultivar responses to low water availabilities without any intermingling with any factors, seeds should be tested by filter paper, where no anoxia (PEG), no ions (Osmotic). Testing seeds germination under water stress not necessarily ensures the capability of

discrimination between susceptible and tolerance cultivars. Germinating of five fababean cultivar seeds at filter paper medium of 300% AWC of the original seed dry weight resulted in significant increases in the percentage of peak germination (20.3; 16.3%) and final germination percentage (33.9; 173.1%) and substantially reduced the germination rate (67.8; 98.4), dead seed percentage (155.2; 255.1%), time to first emergence (42; 76.8%) and duration required for peak emergence (24; 46.7), respectively, as compared to 200% and 100% AWC of tested dry seed weight levels (Abdel and Al-Hamadany, 2007).

Table 2. Regression analysis responses of detected germination parameters to moisture available	oilities.
Tuble = , Regression analysis responses of detected germination parameters to moistare avana,	June 100.

Character	Regression equation	Coeff. Determ. (R ²)
Final Germination (%)	Y = 100 – 0.234 X	36.7
Mean Germ. Time (days)	$Y = 1.67 - 0.061 X + 0.003 X^2 - 0.00002 X^3$	25.5
Germination Energy (%)	Y = 95.05 - 0.533 X	35.3
Germ. Rate (seedling/day)	Y = 52.11 - 0.384 X	35.5
Days for Peak Germination	Y = 3.855 + 0.039 X	32.3
Days for First Emergence	Y = 1.715 0.020 X	41.5
Germ. Rate: Germ. % Ratio	Y = 0.516 - 0.003 X	22.2
Radical Length (mm)	Y = 101.05 – 0.96 X	71.5
Plumule Length (mm)	Y = 60.475 - 0.662 X	59.9
Radical :Plumule Length ratio	$Y = 2.051 - 0.032 X + 0.0008 X^2$	47.1

	Nineveh	Adlib	Baraka
Control	~	t	N
25 % AWC depletion	5	2	7
50 % AWC depletion	3	f	7
75 % AWC depletion	J	(E	2

Fig. 1. Nature of germination and seedling performances of three lentil cultivars in response to four depletion levels of the water available capacity using filter papers.

Cultivar and matric potential interaction

Adlib seed germinated on filter paper saturated by distilled water was the most potent dual interaction treatment. Since this treatment preponderated other interaction treatments in radical length (121.25 mm) (Table, 1; figure, 1-5). Moreover under severe water deprivation (depletion of 75% AWC), Adlib dominance was also unveiled as it gave the highest radical length (51.25 mm), final germination percentage (90%) (Fig. 1, Fig. 4). On the other hand the lowest values of

radical length (6.5 mm) under the highest level of water depletion were confined to Mungbean (Fig. 3). The results suggested unequivocal discrepancies among lentil, vetch and Mungbeans species besides the variations among lentil cultivars under varying moisture levels. These differences might be due to the capabilities of species in conserving the genetic diversity on the mother plants, seed size, and seed location on mother plant.

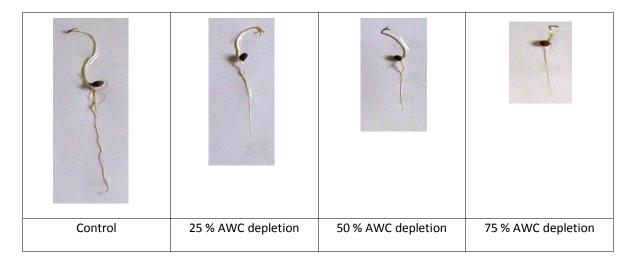


Fig. 2. Nature of germination and seedling performances of Common Vetch in response to four depletion levels of the water available capacity using filter papers.

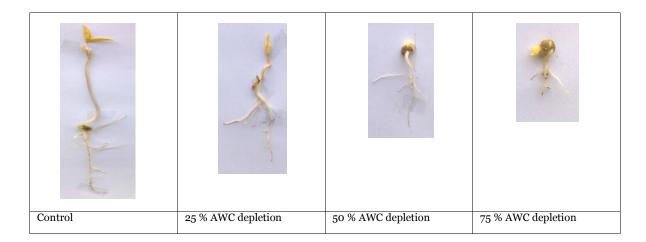


Fig. 3. Nature of germination and seedling performances of Mungbean in response to four depletion levels of the water available capacity filter paper.

I	Baraka	Adlib	NIneveh
o % AWCdepleti			(a
25% AWc depletion			
50% AWC depletion		test	
75% AWC depletion			

Fig. 4. The influence of varying filter paper moisture content on root anatomy of three lentil cultivars (Magnification 7X40).

	Root cap and pical meristems (0% depletion)	Vessels of ring and spiral thickening (25% depletion)
000	Vessel thickening (50% depletion)	Adventurous root formation (75% depletion)

Fig. 5. The influence of varying filter paper moisture content on root anatomy of Common Vetch Local cultivar (Magnification 7X40).

In Chickpea and Maize species, the effect of the water potential to which seeds were exposed on subsequent germination rate on moist filter paper is that at water potentials below a critical value (ψ_{min}), germination rate was constant and was not affected by water potential. At water potentials above ψ_{min} , a linear increase in germination rate with water potential was assumed. For both species the model was fitted by minimizing the sum of squares of the difference between the experimental points and the two-line model. In the virtual osmotic potential (VOP) germination model, ψ_{min} was assumed to be the lowest water potential at which metabolic advancement occurred, and was estimated to be -5.0 and -3.4 MPa for maize and chickpea, respectively. There was no effect of any treatment on the final percentage of seeds that germinated on moist filter paper (Finch-Savage et al., 2005). They also found that cumulative germination curves were recorded at a range of water potentials at 20°C for both maize and chickpea. In both species, the rate of germination was increasingly delayed as water potential decreased. In parallel experiments, germination curves were also recorded at six temperatures between 12.5 and 40°C, and at 11 temperatures between 7 and 43°C for the same seed lots of chickpea and maize, respectively.

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