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Efficiency of Folic Acid in Improving Yield and Fruit Quality of Strawberry

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Abstract

In this research the effect of application of different levels of folic acid and time on some quality and agronomic characteristics of strawberry (cultivar Paros) was investigated. The plants were treated by folic acid with concentrations of 0, 20 and 40 mM at two stages (30 days after planting and first blooming) as a factorial experiment using a randomized complete block design with four replications in the greenhouse conditions. Results showed that fruits of treated plants by folic acid, as compared to the control treatment improved quality and agronomic characteristics. Application of folic acid increased yield, weight of primary and secondary fruits and number of their achenes, total soluble solid, inducing sugar, titratable acidity, anthocyanin, phenol, and vitamin C. The most effective treatment with the best spraying time was 40 mM folic acid at 30 days after planting. The present study is the first evidence that folic acid enhances fruit quality and yield of strawberry.

Keywords: Folic acid; Quality characteristics; Strawberry; Yield and yield components

Abbreviations GAE-Gallic acid equivalents; THF-Tetrahydrofolate; TSS-Total soluble solids

Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is a delicious fruit with high health value and is grown almost all over the world (Cordenunsi *et al.* 2003). Fertilizer management is a vital demand for high quality yield of organically grown strawberry. Foliar nutrition may play an important role in perennial fruit plants. Both quality and quantity of perennial fruit crops have been improved through foliar application of nutrients (Brown *et al*. 1996). Recently, strawberry growers have been mostly interested in growing cultivars for the fresh market. That kind of production is more profitable, but on the other hand it requires more complicated technologies and well-educated workers. High quality of the fruit for the fresh market is an important factor attracting customers and determines their choice and prices. The cost of fruit production for the fresh market needs to be calculated and trials of more efficient methods and technologies should be taken into consideration (Skuien 2003). New environment friendly mineral-organic fertilizers can improve fruit quality and yield of dessert strawberry cultivars (Kholde-Barin and Eslamzadeh 2005). The desired effects are obtained through the activity of fertilizer's components, which very often belong to different groups of natural hormones, elicitors, vitamins, flavonoids, amino acids, etc. (Hakala *et al*. 2003; Nestby *et al*.

2004). Three major components of fruit organoleptic quality are flavor, sweetness and acidity. Several studies have been devoted to strawberry aroma. Fruit with intense flavor also have high titratable acidity and high soluble solids (Hakala *et al*. 2003). Several promising methods have been developed to improve quality and yield characteristics of strawberry fruit. These include fertilization management (Cleber *et al*. 2013), fertilization through irrigation (Hagin *et al*. 2002), cultivar type (Antunes *et al*. 2010), breeding and genetic programs (Hancock *et al*. 2008), chemical composition (Eshghi *et al*. 2012) and cultivation conditions (Hakalam *et al*. 2003).

Folic acid was first isolated in 1941 from spinach (*Spinacia oleracea* L.) and characterized as pteroylmonoglutamic acid, a complex water soluble vitamin B (Cossins 2000; Wang *et al*. 2008). Folic acid helps in the transport of amino acids to appropriate location in the protein chain creation (Kelly 1998) and is involved in methylation of amino acids, DNA and RNA (Lucock *et al*. 1996). Recently, a great attention has been focused on the possibility of using natural and safe substances in order to improve plant growth. In this regard, vitamins such as folic acid have synergistic effects on growth, yield and yield quality of many plant species. These compounds have beneficial effects on catching the free radicals or the active oxygen that are produced during photosynthesis and respiration processes (Foyer *et al*. 1991; Fardet *et al*. 2008). Vitamins have also an auxinic action (Fardet *et al*. 2008). The most familiar vitamins are ascorbic acid (vitamin C) and folic acid (vitamin B9) which are synthesized in higher plants and affect plant growth and development (Samiullah *et al*. 1988). Tetrahydrofolate (THF) and its one-carbon-substituted derivatives (collectively termed folates) are involved in key metabolic functions, including the synthesis of methionine, pantothenate, purines and thymidylate (Oretli 1987). Plants and most microorganisms can synthesize THF de novo, whereas mammals cannot and so require a dietary supply of this soluble vitamin. On the other hand, folic acid (vitamin B9) has become the most prominent of B-complex vitamins despite its essential biochemical function in amino acids metabolism and nucleic acids synthesis (Andrew *et al*. 2000). The beneficial effect of different vitamins on growth and production of field crops was mentioned by many authors such as Rabie and Negm (1992), Mousa *et al*. (1994), Abd El-Messih and Eid (1999), Anton *et al*. (1999), Gamal El-Din-Karima and Reda (2003), Attallah *et al*. (2004), Abd El-Naeem and Abd El-Hakim (2009) and Al-Qubaie (2012).

The objective of this research was to test the effectiveness of folic acid on some quality and yield characteristics of “Paros” strawberry *(Fragaria* × *ananassa* Duch.) plants.

Materials and Methods

This study was carried out in a commercial greenhouse, Jiroft, Iran. Induced and rooted daughter plants of Paros cultivar were planted in greenhouse. The experiment was arranged as factorial based on randomized complete blocks with single plant experimental units and four replications. The treatments were 0, 20 and 40 mM folic acid which applied at 30 days after planting and blooming stage. Then, characteristics such as yield, weight of primary and secondary fruits and number of their achenes, total soluble solids (TTS), inducing sugar, titratable acidity, anthocyanin, phenol, dry weight, vitamin C were measured.

Measurements

TSS, titratable acidity and vitamin C

Total soluble solids, were taken on 5 fruit per plant at harvest using a hand-held refractometer (American Optical Co., Keene, N.H.). Titratable acidity and vitamin C were determined by the method of Basiouny and Woods (1992).

Reducing sugars

For the determination of reducing sugars, one gram of fruit tissue was mixed with 20 ml of 50% ethanol and then incubated in the oven at 60°C for 2h. One ml of the supernatant liquid was mixed with 0.5 ml of 0.1 N hydrochloric acid and boiled for 15 min. This mixture was then mixed with 0.5 ml of 0.1 N sodium hydroxide. One ml of the supernatant liquid was then taken for quantifying the reducing sugars using Hodge-Hofreiter's method (Hodge and Hofreiter 1962).

Anthocyanin content

An aliquot of extract was combined with ethanolic HCl solution (0.25 M) to get a dilution of 1:10. The solution was mixed thoroughly and the absorbance at 520 nm (A520) was read after 5 min, using the ethanolic HCL solution as blank. Total anthocyanin content was determined as follows (Kim *et al*. 2003):

$\%W/W = A/\mathcal{E}L \times MW \times DF \times V/Wt \times 100\%$,

where, A= Absorbance, Ɛ= Cyd-3-glu absorbance (26,900), MW= anthocyanin molecular weight (449.2), DF= dilution factor, V= final volume (mL), Wt = sample weight (mg) and L= cell pathlength (1 cm).

Phenol compound

Measurements were carried out according to a previously published protocol (Arnous *et al*. 2002), employing the Folin-Ciocalteu methodology. Gallic acid was used as the reference standard and results were expressed as mg gallic acid equivalents (GAE) per 100 g of fresh tissue. An aliquot of extract was combined with ethanolic HCI solution (0.25 M) to give a dilution of 1:10. The solution was mixed thoroughly, and the absorbance at 520 nm (A520) was read after 5 min, using the ethanolic HCL solution as blank.

Statistical analysis

Data were analyzed by the analysis of variance and the means were compared ($p \leq 0.05$) by Duncan's multiple rang test. All analyses were performed using SAS program, version 9.1.

Results and Discussion

Analysis of variance revealed that the effect of folic acid and time of application were significant on all measured characteristics including yield, weight of primary and secondary fruits, number of primary and secondary fruits achenes, total soluble solids, inducing sugar, titratable acidity, anthocyanin, phenol and vitamin C (Table 1). This indicates that application of folic acid affected strawberry traits. The time of application also

Table 1. Analysis of variance of quality and agronomic characteristics of strawberry treated with different concentrations of folic acid at 30 days after planting and blooming stages

		Mean Squares										
SOV	df	Yield	WPF	WSF	NPFA	NSFA	TSS	IS	OA	ACNS	Phen	VitC
Treatment (Tr)	2	0.05*	51.02*	38.21*	14001*	7030*	602.7*	1.06*	0.05*	247.9*	1.54*	7.12*
Time 9T	1	0.15*	36.24*	57.01*	3574.0*	2871*	258.5*	0.295*	0.02*	344.1*	0.99*	19.4*
Tr×T	2	0.01*	47.12*	27.39*	2048.0*	1850*	36.1ns	0.52*	0.01*	81.3*	0.27*	0.85*
Error	14	0.005	0.25	0.89	3.11	12	25.1	0.005	0.005	27.0	0.001	0.04
CV%		3.21	2.11	2.01	0.54	5.69	11.35	3.28	10.12	4.05	5.82	8.24

ns= not significant; *,**significant at 0.05 and 0.01 probability levels, respectively
WPF: weight of primary fruit; WSF: weight of secondary fruit; NPFA: number of primary fruit achenes; NSFA: number of secondary fruit achenes; TSS: total soluble solids; IS: Inducing sugars; OA: Organic acid; ACNS: Anthocyanins; Phen: Phenol; VitC: vitamin C

influenced the characters under study. Furthermore, the folic acid × time interaction was significant for all of studied traits. This suggests that the differences among folic acid levels were not similar at different times of application. However, based on Figures 1-9 the interaction was the change in magnitude type rather than crossover. It means that the best folic acid treatment was consistent at both times of folic acid application.

Foliar treatment of strawberry plants with both concentrations of folic acid, significantly improved agronomic characteristics of the treated plants including yield, weight of primary and secondary fruits and number of their achenes as compared to the control treatment. However, this effect was more pronounced when folic acid was sprayed 30 days after planting as compared to the blooming stage and the effect of 40 mM folic acid at 30 days after planting was significantly higher than that of the blooming stage (Figures 1-3). Therefore, the best treatment that improved yield and its components in the strawberry was 40 mM folic acid when sprayed 30 days after planting. Among the many effects of vitamins, those that has attracted recent attention are the yield and quality improvement in many crops. In fact, enhancing growth productivity is of great importance to maximize the yield (Burguieres *et al*. 2007; Budzinski 2008). Figures 1-3 showed that the increase in yield was accompanied by an increase in the weight of primary and secondary fruits and number of their achenes. Several researchers indicated that application of components such as folic acid treatment stimulated transport of amino acids to the appropriate location in protein chain creation (Kelly 1988), regulation of cell division and cell elongation (De-Tullio *et al*. 1999; Naheif and Mohamed 2013), photosynthesis (Andrew *et al*. 2000) and thus yield improvement in different crops (Rabie and Negm 1992; Mousa *et al*. 1994; Abd El-Messih and Eid 1999; Anton *et al*. 1999; Andrew *et al*. 2000; Gamal El-Din-Karima and Reda 2003; Attallah *et al*. 2004; Abd El-Naeem and Abd El-Hakim 2009; Emam *et al*. 2011). Increase in yield could be attributed to the increase in nutrient uptake and or assimilation due to vitamin application (Samiullah *et al*. 1988).

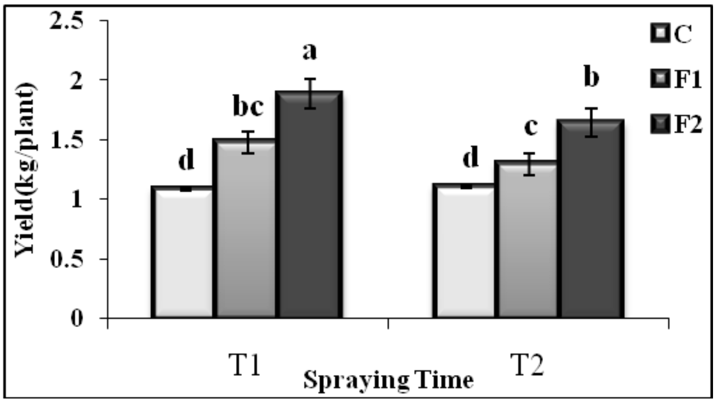

Figure 1. Effect of folic acid on yield of strawberry Paros cultivar (C: control, F1: 20mM Folic acid, and F2: 40 mM Folic acid; T1: 30 days after planting; T2: first blooming. Means followed by same letter are not significantly different at P≤0.05 according to Duncan's multiple range test

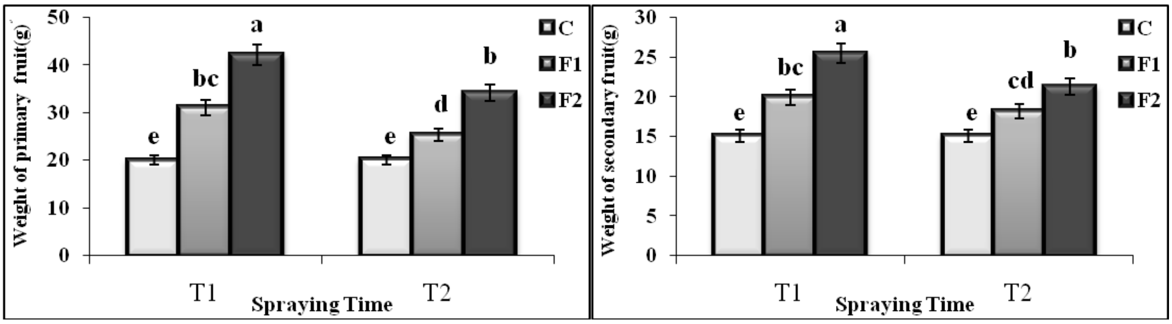

Figure 2. Effect of folic acid on weight fruit of strawberry Paros cultivar (C: control, F1: 20mM Folic acid, and F2: 40 mM Folic acid; T1: 30 days after planting; T2: first blooming. Means followed by same letter are not significantly different at P≤0.05 according to Duncan's multiple range test

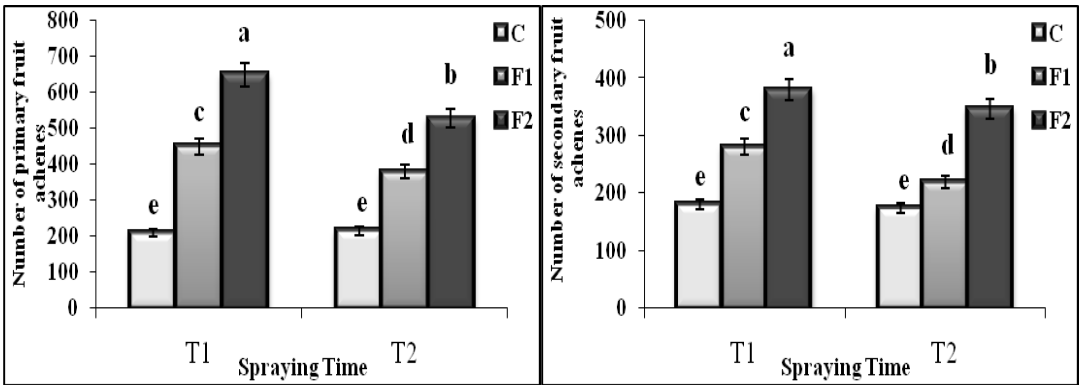

Figure 3. Effect of folic acid on fruit achens number of strawberry Paros cultivar (C: control, F1: 20mM Folic acid, and F2: 40 mM Folic acid; T1: 30 days after planting; T2: first blooming. Means followed by same letter are not significantly different at P≤0.05 according to Duncan's multiple range test

Strawberry fruit is favored for its high percentage of antioxidant components such as ascorbic acid, phenol compounds, titratable acidity and anthocyanin. Antioxidants are molecules that act as free radical scavengers. Most of them are electron donors and react with free radicals to form innocuous end products such as water (Foyer *et al*. 1991; Fardet *et al*. 2008). In addition, natural compounds have been reported to have stronger antioxidant activity than synthetic ones. The quality characteristics of the strawberry fruit under study such as total soluble solids, inducing sugar, titratable acidity, anthocyanin, phenol and vitamin C were improved by both levels of folic acid but the highest amount for all of the quality characters were obtained at 40 mM level and this was more pronounced when foliar application occurred 30 days after planting. Thus, similar to the results for yield and yield components, the best treatment combination in relation to all of the fruit quality characters was the application of 40 mM folic acid 30 days after planting (Figures 4-9). Considerable effort has been carried out to change the quality and antioxidant composition of strawberry and other crops. The increase in the antioxidant components and the improvement in fruit quality and its nutritional value by applying folic acid was reported by Abd El-Naeem and Abd El-Hakim (2009) in vegetable legumes, Burguieres *et al*. (2007) in pes (*Pisum sativum*), Emam *et al*. (2011) in flax (*Linum usitatissimum* L.), and Yavari *et al*. (2008) in strawberry.

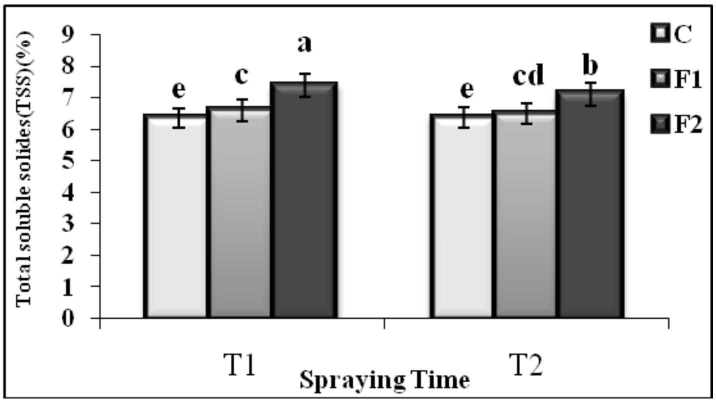

Figure 4. Effect of folic acid on fruit total soluble solids of strawberry Paros cultivar (C: control, F1: 20mM Folic acid, and F2: 40 mM Folic acid; T1: 30 days after planting; T2: first blooming. Means followed by same letter are not significantly different at P≤0.05 according to Duncan's multiple range test

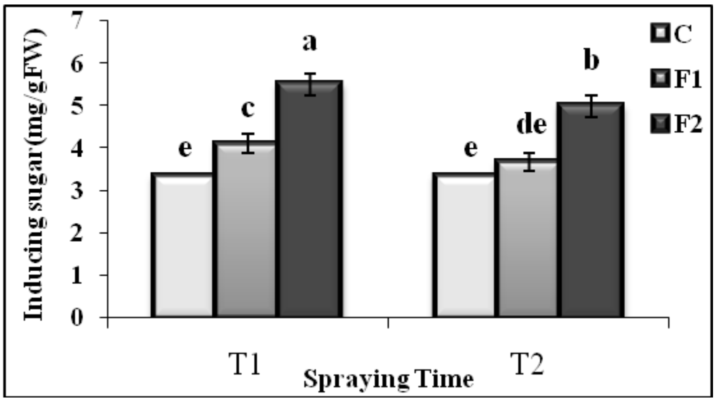

Figure 5. Effect of folic acid on fruit inducing sugar of strawberry Paros cultivar (C: control, F1: 20mM Folic acid, and F2: 40 mM Folic acid; T1: 30 days after planting; T2: first blooming. Means followed by same letter are not significantly different at P≤0.05 according to Duncan's multiple range test

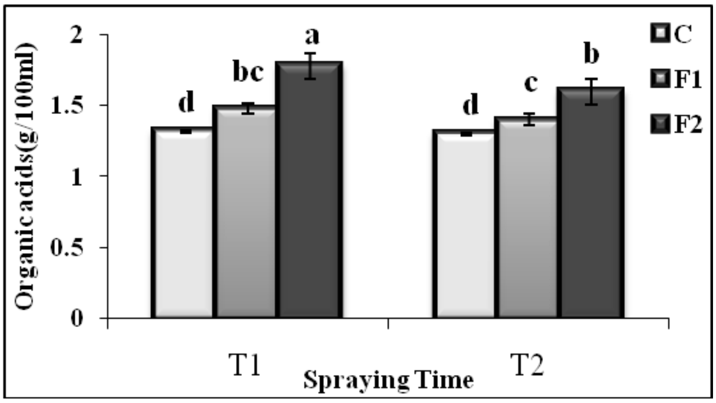

Figure 6. Effect of folic acid on fruit organic acids of strawberry Paros cultivar (C: control, F1: 20mM Folic acid, and F2: 40 mM Folic acid; T1: 30 days after planting; T2: first blooming. Means followed by same letter are not significantly different at P≤0.05 according to Duncan's multiple range test

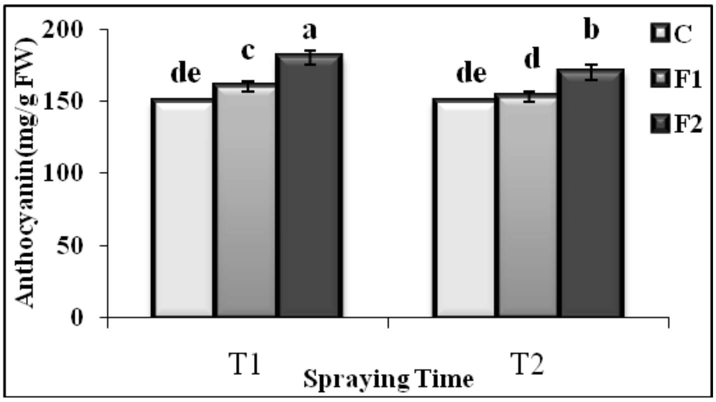

Figure 7. Effect of folic acid on fruit anthocyanin of strawberry Paros cultivar (C: control, F1: 20mM Folic acid, and F2: 40 mM Folic acid; T1: 30 days after planting; T2: first blooming. Means followed by same letter are not significantly different at P≤0.05 according to Duncan's multiple range test

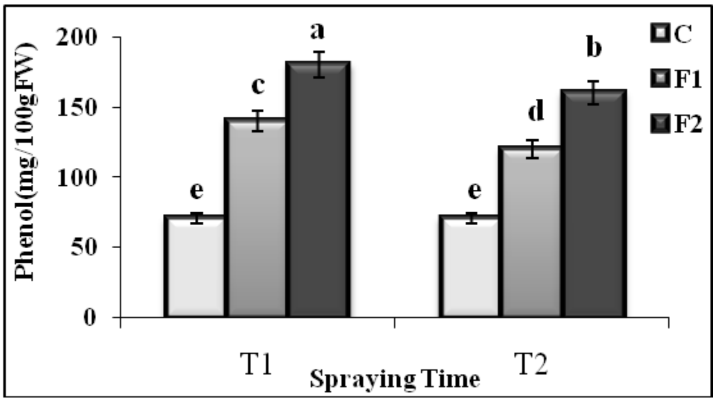

Figure 8. Effect of folic acid on fruit phenol content of strawberry Paros cultivar (C: control, F1: 20mM Folic acid, and F2: 40 mM Folic acid; T1: 30 days after planting; T2: first blooming. Means followed by same letter are not significantly different at P≤0.05 according to Duncan's multiple range test

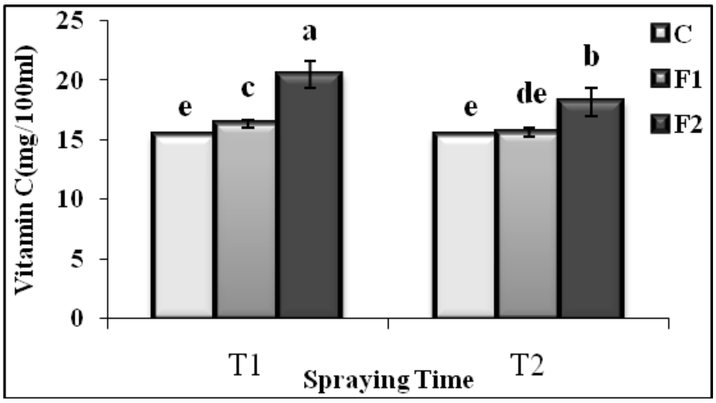

Figure 9. Effect of folic acid on fruit vitamin C of strawberry Paros cultivar (C: control, F1: 20mM Folic acid, and F2: 40 mM Folic acid; T1: 30 days after planting; T2: first blooming. Means followed by same letter are not significantly different at P≤0.05 according to Duncan's multiple range test

Conclusion

Although improving strawberry fruit yield and quality have been reported by many investigators. However, little information is available on the effect of folic acid on antioxidative properties strawberry. In our study, treating strawberry plants, cv. Paros, at 30 days after planting and first blooming with folic acid at 20 and 40 mM resulted in the improvement of yield and yield-related characteristics together with antioxidant compounds. However, application of 40 mM folic acid at 30 days after planting showed the best results. Therefore, this treatment could be recommended if its positive effect remain stable over different environments.

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