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BIOCHEMICAL VARIATION AS INFLUENCED BY BENZYLAMINOPURINE APPLICATION IN WHEAT GENOTYPES UNDER VARIABLE WATER DEFICIT CONDITIONS

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ABSTRACT

The purpose of this study was to investigate the influence of the addition of different concentrations of benzylaminopurine (BAP) in amelioration of the water deficit in wheat genotypes. BAP concentrations were foliarly applied at vegetative and reproductive stages under tillering and boot leaf stage water stress. Water stress given at the tillering stage and the boot leaf stage had significantly reduced the , hill reaction activity, photosynthetic pigments, starch, proteins and significantly induced the sugars, free amino acids, proline in the leaves of the studied genotypes at both 70 DAS and 100 DAS. Foliar application of BAP @100 µg ml-1 given at the vegetative stage under water deficit conditions had showed the stress ameliorative effect. BAP had reduced the detrimental effects of low water availability through stimulating osmotic adjustment in wheat genotypes. BAP have a positive effect on growth factors during water deficit, as stimulating leaf growth and increasing net photosynthetic rates.

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KEY WORDS

Wheat; water stress; tillers; boot leaf; BAP

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[I] INTRODUCTION

Water deficit and salt stresses are global issues to ensure survival of agricultural crops and sustainable food production [1]. Drought is observed in irrigated areas due to insufficient supply of water and canal closure. Water deficit affects every aspect of plant growth by modifying the anatomy, morphology, physiology, biochemistry and finally the productivity of crop. Early grain development stage is more vulnerable to water stress than vegetative growth. The exogenous application of osmoprotectants, growth promoters and antioxidant compounds to plants has been considered as short term solution to alleviate the adverse effects of stress [2]. When the plant tissues were subjected to drought stress, some physiological and biochemical changes occur. Biochemical attributes such as: free proline content, soluble sugar, total protein, decreased phospholipids in the cell membrane [3] and chlorophyll stability can be used as drought tolerance indicators for selecting drought resistant genotypes [4]. Differences in drought tolerance of wheat cultivar were presented by [5]. It was also reported that high yielded variety affected more under stress condition than low yield one [6].

Phytohormones such as cytokinins (CKs) have been reported to reduce the detrimental effects of low water availability through stimulating osmotic adjustment. The foliar spray of osmoprotectants has gained significant ground during the last decade, because it is a shotgun approach to improve stress tolerance in different crops. Phytohormones such as cytokinins (CKs), have been reported to reduce the detrimental effects of low water availability through stimulating osmotic adjustment [7]. Benzylaminopurine (BAP) is also believed to be ideal for exogenous plant application because it is considerably more stable than natural CKs. It is readily taken up by the plant and not degraded by CK oxidase. Water stress tends to accelerate leaf senescence. CKs tend to reduce senescence by maintaining membrane activity [8] and promoting synthesis and inhibiting degradation of protein. This delay in aging is associated with a maintenance of photosynthetic activity [9], thereby enhancing the plant's ability to recover and regrow following water stress. A synthetic CK, BAP has been reported to have a positive effect on some growth factors during water deficit, as stimulating seed germination, leaf growth [10], and increasing net photosynthetic rates.

[II] MATERIALS AND METHODS

2.1. Experimental site

The present investigation was conducted in experimental area of Department of Botany, Punjab Agricultural University, Ludhiana, during

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Rabi season of 2009 and 2010. Ludhiana, representing the Indo-Gangetic alluvial plains, is situated at 30º-54º`N latitude, 75º-45`E longitude and at a mean height of 247 meters above sea level. It is placed in South-Central plain region of Punjab having subtropical and semi-arid climate.

2.2. Experimental treatments and design

The two genotypes PBW 343 and PBW 527 were raised and treatments were allotted in split plot design. Each treatment was replicated thrice.

2.3. Treatments

T1–Untreated control
T2 –Water-deficit at tillering stage (at 50% level using tensiometer)
T3– Water-deficit at boot leaf stage (at 50% level using tensiometer)
T4 –T2+50 µg ml-1 BAP at vegetative stage
T5 –T2+100 µg ml-1 BAP at vegetative stage
T6 –T2+50 µg ml-1 BAP at vegetative and post-anthesis stage
T7 –T2+100 µg ml-1BAP at vegetative and 50µg ml-1at post-anthesis stage
T8 –T3+50 µg ml-1 BAP at vegetative stage
T9–T3+100 µg ml-1 BAP at vegetative stage

2.4. Crop husbandry

The pre-sowing irrigation (75 mm) was applied, prior to sowing; the soil of the replications was carefully leveled to ensure even distribution of water. All the required field management practices were followed according to the specifications laid out in the "Package of Practices for Rabi crops 2009-2010"a handbook of Punjab Agricultural University, Ludhiana. Recommended dose of fertilizers was applied at the time of sowing. Seeds were sown, after soil become in conditions of sowing, each treatment was allotted rows of four meters length. Inter row and inter plant distance was maintained at 20 cm and 8.5 cm respectively. Weeding and hoeing were carried out manually to keep the crop free from weeds throughout the growth period. Foliar spray of BAP at vegetative stage was given at 60 DAS and at reproductive stage was given at 90 DAS. The observations for the biochemical parameters were recorded after 70 DAS and 100 DAS.

2.5. Data collection

The observations for the biochemical parameters (plant pigments [11], hill reaction activity [12], protein [13], free amino acids [14], proline [15], starch [16], and total soluble sugars [17] were recorded.

2.6. Statistical analysis

For the biochemical estimations the data of genotypes sown under variable water deficits were evaluated and Analysis of Variance (ANOVA) was done.

[III] RESULTS AND DISCUSSION

3.1. Photosynthetic pigments

It was clear from **Supplementary Table–1** that there was an inverse proportional relationship between increasing the severity of drought on one hand and contents of leaves of chlorophyll a, b and total pigments on the other hand. Water stress had significantly decreased the chlorophyll-a content. Water stress at tillering stage decreased the chlorophyll a up to much more extent than that of the water stress given at boot leaf stage. Foliar application of BAP had significantly increased the chlorophyll a content under water stress conditions. From among the genotypes, increase in chlorophyll-a in PBW 527 was more than PBW 343 when it was applied with BAP under the stress conditions. There was overall decrease in chlorophyll b concentration under water deficit conditions. Decreased in the concentration of chlorophyll-b was more in PBW 527 as compared to the PBW 343 under water stress. Foliar application of BAP significantly increased the chlorophyll b under tillering water stress. BAP at its higher concentration was found to be better to increase the chlorophyll-b under water deficit conditions. In the present study water stressed plants showed significant decrease in the total chlorophyll. Foliar application of BAP increased the total chlorophyll under stress conditions.

3.2. Hill reaction activity

The rate of hill activity may be limited by almost all adverse environmental factors. From among the genotypes, PBW 527 had more hill reaction activity than that of the PBW 343 under full turgor conditions. Water stress had significantly decreased the hill activity when it was applied at the stages of the growth of wheat plants i.e. (tillering as well as boot leaf stage). Results pointed out that the decrease was more pronounced when water stress was applied at the tillering stage in PBW 343 and at the boot leaf stage in PBW 527 **[Supplementary Table–2]**. Foliar application of BAP at its higher concentration had significantly increased the hill activity under stress which was given at the (tillering and boot leaf stage) when sprayed at the vegetative stage. But the higher application in addition to the lower application at post anthesis i.e. (T7) was found to be more effective to increase the hill activity under tillering water stress conditions in both the genotypes.

3.3. Proline

Proline is the most common osmolyte accumulated in water stress conditions and the accumulation of these compounds is thought to represent an important adaptive response to drought stress. Proline accumulation is also correlated to the increase in total catabolic amino acids and sugar during stress. The proline level in control plant is also found to vary between varieties of the same crop grown under same physiological, soil and environmental conditions i.e. among the control plants, some cultivars show high values whereas others find low. Results showed that PBW 527 had more proline accumulation than that of the PBW 343 **[Supplementary Table–3]** at 100 DAS. Water deficit had significantly increased the proline content at 70 DAS as well AS 100 DAS. In the present study foliar application of BAP at 60 DAS and 100 DAS had significantly reduced the proline concentration under the stress conditions whether the stress was applied at the tillering stage or the boot leaf stage. BAP had significantly decreased the proline when it was applied at the higher concentration under the tillering water stress as well as under boot leaf water stress at both 70 DAS and 100 DAS in the leaves of studied genotypes.

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3.4. Reducing sugars, Starch, Proteins and Total amino acids

Reducing sugar, proteins and free amino acid values varied significantly among the wheat genotypes. In comparison among the genotypes, PBW 527 possessed high soluble sugar content as compared to the PBW 343 under the control conditions. It was evident that the values of soluble sugars extracted from the leaves in the presently studied genotypes of *Triticum* increased progressively by increasing the deficit period. The increase in sugar content was found to be more drastic when the water stress was given at the boot leaf stage. The increase in level of reducing sugar under water stress may also be ascribed to an increase in starch hydrolysis. Results revealed that foliar application of BAP had significantly increased the sugar content when sprayed at the vegetative stage under tillering water stress as compared to the control. The increase was more pronounced in the PBW 527 **[Supplementary Table–4]**.

Two wheat genotypes chosen on the basis of their different drought tolerance were grown in field and subjected to drought at two stages of development (tillering and the boot leaf stage). Highly significant differences were detected between watered and stressed plants for starch content in both the genotypes, proved that the treatment applied leads to a real water deficit.

The both genotypes had showed the different behavior for starch content values. Results revealed significant variations in the values of starch among the two wheat genotypes under consideration. Drought stress decompose starch and fade it from the plant. It was earlier reported that drought stress causes many changes in the amount of plants carbohydrate and it become clear that with increasing drought stress on leaves, the amount of starch decrease. Water stress had significantly decreased the starch content in flag leaves at 70 DAS and 100 DAS. But the decrease was more recorded under tillering stress as compared to the stress given at the boot leaf water stress.

Results showed that foliar application of BAP had significantly decreased the starch in both the genotypes in the flag leaves at 70 DAS and 100 DAS as compared to the control under the stress conditions whether the stress was given at the tillering stage or at the boot leaf stage.

Results showed that free amino acids were increased at the stage of maturity in PBW 527, but decrease in PBW 343 at the stage of maturity **[Supplementary Table–5]**. Results pointed out that water stress had significantly increased the free amino acid. But the increase was more under boot leaf stress in both the genotypes. The properties of compatible solutes facilitate the maintenance of favorable turgor pressure during water stress and in addition may serve as protective agents by stabilizing proteins. Results showed that foliar application of BAP had significantly increased the free amino acids in both the genotypes as compared to the control under the stress conditions at 70 DAS as well as at 100 DAS.

In leaves of studied genotypes protein content was estimated at two stages of growth and results showed the significant differences between the genotypes under stress treatments. From among the genotypes PBW 527 had more proteins as compared to the PBW 343 at both 70 DAS and 100 DAS. Exposing wheat plants to osmotic stress decreased total protein concentration relative to the control treatments in both the genotypes. The decrease was more pronounced when the water stress was applied at the boot leaf stage. Results showed that foliar application of BAP had significantly increased the protein content in both the genotypes as compared to the control under the stress conditions. Protein content was higher at 70 DAS and lower at 100 DAS in both the genotypes.

[III] DISCUSSION

Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers [18]. A reduction in chlorophyll content under drought was found presently. It was also reported that BAP application reduced the reduction in chlorophyll content and maintained it at higher level in the leaves [19]. Reduction of starch was the result of amylase activity that increased soluble sugar [20]. Water stress affects the conversion of sucrose in to starch [21]. Water stress reduced starch content in the shoots of tolerant seedlings as compared to the sensitive ones, but increased sucrose content in the shoots and roots of tolerant seedling, indicating their protective role during stress conditions [22]. This increment was significant at boot leaf stage. Previous data showed that level of these metabolites increased over control at 1.0 mg l-1 CK treatment after which variations were non significant [23]. The decrease in the water availability for transport-associated process leads to change in the concentration of many metabolites followed by distribution in amino acid and carbohydrate metabolism and increase in synthesis of compatible solutes, such as amino acids. Therefore, free amino acid seem to be additional to proline accumulation for deciding tolerance in a given crop species. Compatible solutes are synthesized in response to osmotic stress and can occur at high intracellular concentrations without hindering normal cellular metabolism. Increased protein content as well as depletion of amino acids in the grains of kinetin and ethrel treated plants indicate, efficient incorporation of amino acids into proteins. Kinetin had been reported to maintain higher rate of protein synthesis [24]. Application of BAP on younger and older leaves enhanced the soluble protein content except at few stages [25] are also reported in increasing in protein level under influence of BAP in beans.

[IV] CONCLUSION

From this study it was clearly observed that at the stages of drought and subsequent rehydration compounds with cytokinin (BAP) activities were found the most efficient protectors, enhancing a less pronounced decrease in the intensity of photosynthetic efficiency. BAP is a regulator of leaf senescence and their effects is dramatic particularly when sprayed directly

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on the intact plants. BAP protects the cell membranes and the photosynthetic machinery from oxidative damage by delay of senescence. BAP stimulated chloroplast differentiation and inclusion of BAP induced the formation of greater numbers of chloroplasts in the leaves. Thus BAP promoted development of more leaf area and greater plant survival rates under varied water deficit conditions. A higher amount of amino acids and proteins were observed, accumulation of these metabolites in foliar cells may contribute towards dry mass distribution and osmotic adjustment Thus foliar application of BAP @100 µg ml-1 given at the vegetative stage under water deficit conditions had showed the stress ameliorative effect.

CONFLICT OF INTERESTS

Authors declare no conflict of interests.

FINANCIAL DISCLOSURE

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SUPPLEMENTARY TABLES (As supplied by authors)

Supplementary Table: 1. Influence of BAP on chlorophyll a, chlorophyll b and total chlorophyll (mg $^{-1}$ gm fresh weight) content in wheat (*Triticum aestivum* L.) genotypes under water stress

GENOTYPE TREATMENTS	CHLOROPHYLL A				CHLOROPHYLL B				TOTAL CHLOROPHYLL			
	PBW343		PBW527		PBW343		PBW527		PBW 343		PBW527	
	70DAS	100DAS	70DAS	100DAS	70 DAS	100DAS	70DAS	100DAS	70DAS	100DAS	70DAS	100DAS
T_1-Untreated control	0.7210	0.6200	0.7307	0.7133	0.2639	0.2212	0.2919	0.2442	0.9849	0.8412	1.0226	0.9575
T_2 –Water-deficit at tillering stage	0.3810	0.5844	0.4203	0.6217	0.2396	0.2168	0.2515	0.2075	0.6206	0.8012	0.6718	0.8292
T_3 Water-deficit at boot leaf stage	0.7216	0.3448	0.7135	0.4222	0.2837	0.1914	0.2630	0.1877	1.0053	0.5362	0.9765	0.6099
CD5%	**0.0767**	**0.0623**	**0.0700**	**0.0657**	**0.0093**	**0.0065**	**0.0098**	**0.0128**	**0.0866**	**0.0688**	**0.0798**	**0.0785**
T_4-T_2+50 µg ml-1BAP at vegetative stage	0.4646	0.5864	0.5073	0.6436	0.2613	0.2168	0.2575	0.2088	0.7259	0.8032	0.7648	0.8524
T_5-T_2+100 µg ml-1BAP at vegetative stage	0.5891	0.5879	0.5363	0.6476	0.2773	0.2074	0.2632	0.2119	0.8664	0.7953	0.7995	0.8595
T_6-T_2+50 µg ml-1 BAP at vegetative and post-anthesis stage	0.3933	0.6059	0.5086	0.6514	0.2618	0.2033	0.2612	0.2150	0.6551	0.8092	0.7698	0.8664
T_7–T_2+100 µg ml-1BAP at vegetative and 50 µg ml-1at post-anthesis stage	0.4652	0.6077	0.5360	0.6535	0.2739	0.2204	0.2681	0.2160	0.7391	0.8281	0.8041	0.8695
CD5%	**0.0618**	**0.0501**	**0.0563**	**0.0529**	**0.0072**	**0.0052**	**0.0077**	**0.0049**	**0.0697**	**0.0553**	**0.0643**	**0.0578**
T_8-T_3+50 µg ml-1BAP at vegetative stage	0.7859	0.4422	0.7409	0.4230	0.3085	0.1960	0.2658	0.1947	1.0944	0.6382	1.0067	0.6177
T_9-T_3+100 µg ml-1BAP at vegetative stage	0.7868	0.4518	0.7546	0.4269	0.3255	0.1963	0.2813	0.1946	1.1123	0.6481	1.0359	0.6215
CD5%	**0.0110**	**0.0151**	**0.0195**	**0.0139**	**0.0094**	**0.0087**	**0.0128**	**0.0128**	**0.0204**	**0.0238**	**0.0323**	**0.0267**

Supplementary Table: 2. Influence of BAP application on Hill reaction activity (mg chlorophyll^{-1} hr^{-1}) in wheat (*Triticum aestivum* L.) genotypes under water stress

Genotype	PBW 343		PBW 527	
Treatments	**70 DAS**	**100DAS**	**70 DAS**	**100 DAS**
T_1-Untreated control	0.2573	0.2292	0.3080	0.3148
T_2 –Water-deficit at tillering stage	0.1111	0.2158	0.1577	0.2630
T_3 Water-deficit at boot leaf stage	0.2573	0.1482	0.2929	0.1150
CD5%	**0.0329**	**0.0182**	**0.0339**	**0.0449**
T_4-T_2+50 µg ml-1 BAP at vegetative stage	0.1369	0.2187	0.1685	0.2590
T_5-T_2+100 µg ml-1 BAP at vegetative stage	0.1568	0.2225	0.1804	0.2794
T_6 -T_2+50 µg ml-1 BAP at vegetative and post-anthesis stage	0.1340	0.2210	0.1688	0.2825
T_7 –T_2+100 µg ml-1 BAP at vegetative and 50µgml-1at post-anthesis stage	0.1581	0.2258	0.1834	0.3073
CD5%	**0.0136**	**0.0137**	**0.0174**	**0.0553**
T_8-T_3+50 µg ml-1 BAP at vegetative stage	0.2593	0.1746	0.3039	0.1386
T_9-T_3+100µg ml-1 BAP at vegetative stage	0.2631	0.2030	0.3176	0.1595
CD5%	**0.0493**	**0.0858**	**0.0256**	**0.0381**

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Supplementary Table: 3. **Influence of BAP application on Free Proline (mg $^{-1}$ gm fresh weight) in wheat (*Triticum aestivum* L.) genotypes under water stress**

Genotype	PBW 343		PBW 527	
Treatments	**70 DAS**	**100 DAS**	**70 DAS**	**100 DAS**
T_1.Untreated control	2.554	3.083	2.219	3.356
T_2 –Water-deficit at tillering stage	6.163	3.142	4.050	4.244
T_3 Water-deficit at boot leaf stage	2.780	5.959	2.277	7.371
CD5%	**0.8129**	**0.6481**	**0.4132**	**0.9075**
T_4-T_2+50 µg ml-1BAP at vegetative stage	4.138	3.334	3.988	3.625
T_5-T_2+100 µg ml-1BAP at vegetative stage	3.679	3.212	3.818	3.431
T_6 -T_2+50 µg ml-1 BAP at vegetative and post-anthesis stage	4.009	3.134	2.841	4.187
T_7 –T_2+100 µg ml-1BAP at vegetative and 50µgml-1at post-anthesis stage	3.515	3.062	2.659	3.916
CD5%	**0.6544**	**0.0603**	**0.3326**	**0.6319**
T_8-T_3+50 µg ml-1BAP at vegetative stage	2.454	4.959	2.185	4.953
T_9-T_3+100µg ml-1BAP at vegetative stage	2.096	4.208	2.118	4.271
CD5%	**0.0335**	**0.6469**	**0.0420**	**0.9055**

Supplementary Table: 4. **Influence of BAP application on Sugar and starch (mg $^{-1}$ gm fresh weight) in wheat (*Triticum aestivum* L.) genotypes under water stress**

GENOTYPE TREATMENTS	SUGARS				STARCH			
	PBW343		PBW527		PBW343		PBW527	
	70 DAS	100DAS	70DAS	100DAS	70 DAS	100DAS	70DAS	100DAS
T_1.Untreated control	11.044	17.416	14.245	18.194	5.695	4.053	6.871	4.551
T_2 –Water-deficit at tillering stage	13.017	18.088	17.773	19.032	5.059	3.333	6.530	3.931
T_3 Water-deficit at boot leaf stage	11.424	22.052	15.011	19.716	5.563	3.603	6.904	4.111
CD5%	**0.4449**	**1.203**	**1.474**	**0.3433**	**0.1438**	**0.1423**	**0.1400**	**0.0844**
T_4-T_2+50 µg ml-1BAP at vegetative stage	13.376	18.801	17.917	20.012	4.657	3.153	5.680	3.351
T_5-T_2+100 µg ml-1BAP at vegetative stage	13.444	18.934	18.581	20.068	4.741	2.863	5.801	3.341
T_6-T_2+50 µg ml-1 BAP at vegetative and post-anthesis stage	13.378	21.128	18.496	19.869	4.693	2.953	5.724	3.661
T_7–T_2+100 µg ml-1BAP at vegetative and 50µgml-1at post-anthesis stage	13.457	21.266	18.620	20.032	4.717	2.813	5.856	3.621
CD5%	**0.0682**	**0.9689**	**0.8736**	**0.2765**	**0.1155**	**0.1145**	**0.1123**	**0.0679**
T_8-T_3+50 µg ml-1BAP at vegetative stage	11.476	22.940	15.036	20.612	5.017	2.843	6.175	3.781
T_9-T_3+100 µg ml-1BAP at vegetative stage	11.976	23.332	15.046	20.640	5.065	2.783	6.351	3.511
CD5%	**0.0444**	**0.0773**	**0.0023**	**0.3434**	**0.1435**	**0.1423**	**0.1403**	**0.0846**

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Supplementary Table: 5. **Influence of BAP application on total amino acids and Protein content (mg $^{-1}$ gm fresh weight) in wheat (*Triticum aestivum* L.) genotypes under water stress**

GENOTYPE TREATMENTS	FREE AMINO ACIDS				PROTEIN CONTENT			
	PBW343		PBW527		PBW343		PBW527	
	70 DAS	100DAS	70DAS	100DAS	70 DAS	100DAS	70DAS	100DAS
T_1-Untreated control	11.044	17.416	14.245	18.194	6.450	5.639	7.724	5.257
T_2 –Water-deficit at tillering stage	13.017	18.088	17.773	19.032	5.039	5.154	6.527	5.008
T_3 Water-deficit at boot leaf stage	11.424	22.052	15.011	19.716	6.374	4.273	7.752	4.166
CD5%	**0.4449**	**1.203**	**1.474**	**0.3433**	**0.0932**	**0.1097**	**0.0432**	**0.0553**
T_4-T_2+50 µg ml-1BAP at vegetative stage	13.376	18.801	17.917	20.012	6.221	5.219	7.621	5.029
T_5-T_2+100 µg ml-1BAP at vegetative stage	13.444	18.934	18.581	20.068	6.288	5.231	7.677	5.044
T_6-T_2+50 µg ml-1 BAP at vegetative and post-anthesis stage	13.378	21.128	18.496	19.869	6.249	5.355	7.649	5.064
T_7–T_2+100 µg ml-1BAP at vegetative and 50µgml-1at post-anthesis stage	13.457	21.266	18.620	20.032	6.268	5.426	7.687	5.084
CD5%	**0.0682**	**0.9689**	**0.8736**	**0.2765**	**0.0753**	**0.0879**	**0.0356**	**0.0441**
T_8-T_3+50 µg ml-1BAP at vegetative stage	11.476	22.940	15.036	20.612	6.546	5.438	7.949	5.217
T_9-T_3+100 µg ml-1BAP at vegetative stage	11.976	23.332	15.046	20.640	6.613	5.456	8.099	5.247
CD5%	**0.0444**	**0.0773**	**0.0023**	**0.3434**	**0.0929**	**0.0022**	**0.0458**	**0.0006**

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